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COMPARATIVE UTILIZATION AND COST BENEFIT OF FEEDING THREE NOVEL INGREDIENTS TO BROILER CHICKENS

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Abstract

This study was conducted to determine the effect of feeding three differently processed discarded vegetable-bovine blood-rumen content mixture on nutrient digestibility and cost benefits of broiler chickens. A total of 1,080 day-old Marshal broiler chickens were fed diet containing discarded vegetable-fresh bovine blood-fresh rumen digesta (P1), discarded vegetable-ensiled bovine blood-fresh rumen digesta (P2) and discarded vegetable-fresh bovine blood-ensiled rumen digesta (P3) at three levels of inclusion (0, 3 and 6%) at the starter phase while 540 of the birds were transferred and redistributed in the finisher phase of the experiment. Performance indices of birds were taken weekly. Data obtained on nutrient digestibility and cost benefits were subjected to 3 x 3 factorial arrangement in a completely randomized design. Results showed birds on P3 had higher crude protein, ether extract and Nitrogen free extract digestibilities than birds fed P1 and P2 at the starter phase. At the finisher phase, digestible ether extract and crude fibre were highest in birds fed P3. Also, digestible nitrogen-free extract was best at 6 % level of inclusion. Increasing level of inclusion of the three processing methods revealed an increase in value of digestible ether extract of the birds except at P3. The best Cost/kg weight gain value was obtained in birds at 0% level of inclusion while values for 3 and 6 % level of inclusion were statistically (P>0.05) similar at the finisher phase. There was also a decrease in the cost/kg weight gain values in P2 and P3 as the inclusion level increased from 0 - 30% at the finisher phase. This study revealed that, in terms of cost of production and for enhanced growth performance, broiler chickens could be fed diets containing discarded vegetable-fresh bovine blood-ensiled rumen digesta (P3) up to 6% level of inclusion.

Keywords: Nutrient digestibility, cost benefits, novel ingredients, discarded vegetable, bovine blood, rumen content.

ETUDE COMPARATIVE DE L'UTILISATION ET RAPPORT COÛT-BÉNÉFICE D'UNE ALIMENTATION DE POULETS DE CHAIR À BASE DE TROIS INGRÉDIENTS NON TRADITIONNELS

Résumé

La présente étude a été réalisée dans le but de déterminer l'effet d'une l'alimentation à base de trois mélanges traités différemment (composés de déchets de légumes, de sang bovin et de contenu du rumen) sur la digestibilité et le rapport coûts-bénéfices des poulets de chair. Au total, 1080 poulets de chair Marshal âgés d'un jour ont été soumis à des régimes consistant en mélanges de déchets de légumes / sang bovin frais / digesta frais de rumen (P1) ; déchets de légumes / sang bovin ensilé / digesta frais de rumen (P2) ; et déchets de légumes / sang bovin frais / digesta de rumen ensilés (P3), à trois niveaux d'inclusion (0 ; 3 ; et 6%) lors de la phase de démarrage, tandis que 540 de ces oiseaux ont été transférés et répartis à nouveau durant la phase de finition de l'expérience. Les indices de performance des oiseaux ont été enregistrés chaque semaine. Les données obtenues sur la digestibilité des nutriments et le rapport coûts-bénéfices ont été soumises à un dispositif factoriel 3 x 3 dans un schéma complètement aléatoire. Les résultats ont montré que les oiseaux au régime P3 avaient des taux plus élevés de digestibilité des protéines brutes, des extraits d'éther et des extraits exempts d'azote par rapport aux oiseaux soumis aux régimes P1 et P2 durant la phase de démarrage. Lors de la phase de finition, le niveau de digestibilité de l'extrait d'éther et des fibres brutes était plus élevé chez les oiseaux nourris au régime P3. En outre, le taux digestibilité de l'extrait exempt d'azote était au mieux au niveau d'inclusion de 6 %. L'augmentation du niveau d'inclusion

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dans les trois méthodes de traitement a révélé une augmentation du taux de digestibilité de l'extrait d'éther chez les oiseaux, à l'exception du régime P3. La meilleure valeur coût / kg gain pondéral a été obtenue chez les oiseaux au niveau d'inclusion de 0%, tandis que les valeurs pour les niveaux d'inclusion 3% et 6% étaient statistiquement (P> 0,05) similaires durant la phase de finition. L'on a également noté une diminution des valeurs coût / kg de gain pondéral dans les régimes P2 et P3 au fur et à mesure de l'augmentation du taux d'inclusion de 0% à 30% lors de la phase de finition. Cette étude a révélé que, en termes de coût de production et pour une meilleure performance de croissance, les poulets de chair pourraient être nourris avec des régimes composés de déchets de légumes, sang bovin frais et digesta de rumen ensilé (P3) jusqu' au niveau d'inclusion de 6%.

Mots-clés : digestibilité des nutriments, rapport coûts-bénéfices, ingrédients non traditionnels, déchets de légumes, sang bovin, contenu de rumen

Introduction

Poultry is considered to be means of support and a way of achieving an assured level of economic freedom in Nigeria. The poultry industry forms the core of the Nigerian economy in supplying the needed necessary protein intake for the populace. Expansion of the poultry industry depends to a large extent on the availability of good quality feed in sufficient quantity (good quality) at a price producers can afford. This is particularly true of the intensive poultry enterprises whose performance depends entirely on the use of balance concentrate rations.

The spiralling cost and availability of these ingredients serves to impair the process of feeding and consequently affecting poultry meat output (Esonu *et al.,* 2001, Ekunseitan *et al.,* 2012). The reduction in quality confirms the economics of trade which showed that reduced quality and low-priced feeds did not essentially give higher income and greater profit (Ekunseitan, 2012, Onibi *et al.,* 1999). This high cost of feed is due mainly to scarcity of feed ingredients as a result of the ban on importation of feed ingredients and low level of production of such ingredients or alternative locally.

The major reason for the escalating cost is the unending competition between humans and livestock for most of these ingredients (Omle and Tewe, 1991). Realistically too, another solution to the problem in Nigeria and globally lies in increased utilization of alternative sources of ingredients referred to as unconventional feed resources (UCFR) and the continuing investigation and economic assessment of the potential of UCFR as animal feedstuffs (Fetuga and Tewe, 1985). There are many agro-industrial by-products and wastes, which are not directly utilizable by man in this country.

Bovine rumen is an abundant waste material (Adeniji, 1996) which has become an environmental toxic waste when dumped in open fields of lands leading to contamination and serving as breeding grounds for flies (Ekunseitan *et al.,* 2012). Bovine blood has been used time in memorial in poultry feed but with its use restricted by some limitations. These limitations include odour impact on finished feed and reduced intake of feed by animals. A mixture of the three test material will serve to complement one another in terms of increasing the available protein contained in bovine blood, increasing the acceptability and possibly serving as a good alternative source of protein in periods of scarcity of conventional ingredients.

The need to grow feed, feedstuffs and alternative ingredients should form the core of activities centred in the progress of the poultry industry. The various investigations on the use of cheaper industrial by-products and farm wastes in poultry nutrition are being intensified with the aim of determining their efficiency of utilization for growth and production. Therefore the study was conducted to check the response of birds to processed ingredients.

Materials and Method

Area Description

The study was carried out at Alao Farms, Tanke-Akata, Ilorin, Kwara state, Nigeria.

The site (Ilorin) is on longitude 4o 35'N and latitude 8o 30'E, elevation 352m and altitude 1.14Km (Google earth, 2014).

Experimental birds and management

A total of 1080, day old Hubbard strain broiler were selected, weighed and randomly allotted to nine treatments comprising of 3 processing methods and 3 levels of inclusion of mixtures. Each treatment was sub-divided into three replicates of 40 birds each. The inclusion of the three differently processed mixtures in the diet meal was at 3 levels: 0, 3 and 6 %. At the finisher phase, a total of 540 birds from the broiler starter population were re-allotted in the same factorial experimental layout to obtain 9 dietary treatment groups of 60 birds each and 3 replicates of 20 birds each.

Collection and Processing of Test Ingredients

Fresh bovine blood and rumen content were collected from several cattle at a slaughter slab via placing a bowl below the neck of decapitated cattle while rumen content was obtained immediately the gut was split. The dietary mixtures were then processed as described by Ekunseitan *et al.,* 2013: Discarded vegetable, fresh bovine blood and fresh rumen digesta (P1), Discarded vegetable, ensiled bovine blood and fresh rumen digesta (P2) and Discarded vegetable, fresh bovine blood and ensiled rumen digesta (P3)

Preparation of Diets

The Processed ingredients were used in the formulation of nine experimental diets to replace soybean meal in diet to meet the nutrient requirements of birds at the starter and finisher phases of growth. The crude protein and metabolizable energy (Kcal/Kg) contents of the feed was within the recommended range (NRC, 1994). The diet composition is presented in Tables 1 and 2.

Nutrient Digestibility

Apparent digestibilities of crude fibre, crude protein, ether extract and nitrogen free extracts of the diets was determined at the end of 3rd and 7th week of the experiment by total collection method (Bourdillion *et al.,* 1990). Three bird per replicate were randomly selected and housed in clean and disinfected individual cages for a 3-day adaptation period, one day fasting to empty their digestive tracts and daily excreta collection period for three consecutive days. During the adaptation period, feed and water was supplied ad libitum. At the termination of 24 hours starvation period, the birds were given known quantities of feed. Droppings were collected on 24 hour basis for three days, the droppings were dried and ground for subsequent analyses to determine crude fibre, crude protein, ether extract and ash digestibilities (AOAC, 2000).

$$\text{Apparent nutrient digestibility} = \frac{(\text{Nutrient in feed intake} - \text{Nutrient in faecal output})}{(\text{Nutrient in feed intake})} \times 100$$

Cost:Benefit analysis

The prevailing market prices of the ingredients at the time of the study was used to calculate the cost of 1kg feed consumed and the cost of 1kg feed consumed/weight gain.

Data Analysis

Data collected were arranged in a 3 x 3 factorial arrangement in a completely randomized design. Significant differences among treatment means were determined using Duncan Multiple Range Test (Duncan, 1955) as contained in SAS (2011) package.

Experimental Model:

$\gamma ijk = \mu + \tau i + \beta j + (\tau\beta)ij + \varepsilon ijk$

Where:

γijk = Observed value of the dependent variable (output)

μ = Population mean (Overall mean)

τi = Effect of Processing methods (i = P1, P2, P3)

βj = Effect of levels of inclusion (j = 0, 3 and 6%)

$(\tau\beta)ij$ = Interaction Processing methods and levels of inclusion

εijk = Random error

Table 1: Composition (%) of Broiler starter diets

	1	2	3	4	5	6	7	8	9
	0%	3%	6%	0%	3%	6%	0%	3%	6%
Ingredient									
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Wheat offal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Groundnut Cake	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	17.50
Soybean meal	20.00	17.00	14.00	20.00	17.00	14.00	20.00	17.00	14.00
P 1	-	3.00	6.00	-	-	-	-	-	-
P 2	-	-	-	-	3.00	6.00	-	-	-
P 3	-	-	-	-	-	-	-	3.00	6.00
Fish meal (72%)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
*Vit./Min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Palm-Oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Determined analysis									
Crude protein (%)	21.09	20.97	21.16	21.09	20.71	20.59	21.09	20.70	20.69
Crude fibre (%)	4.42	4.48	4.53	4.42	4.63	4.82	4.42	4.60	4.77
Ether extract (%)	6.78	4.23	6.03	6.78	4.43	4.71	6.78	4.81	7.41
**ME(MJ/kg)	12.60	11.39	11.74	12.60	11.54	11.43	12.60	11.65	12.07

**Premix composition per kg diet:Vit A :400000IU,Vit D:80000IU,Vit E:40000ng,Vit k3:800mg,Vit B1:1000MG,Vit B2:6000mg, Vit B6:500mg,VitB12:25mg, Niacin:6000mg, Panthothenic acid:2000mg, Folic acid: 200mg, Biotin:8mg, Manganese:300000g, Iron:8000mg, Zinc:20000g, Cobalt:80mg, Iodine:400mg, Selenium:40mg, Choline:800000g*

***Estimated using the formula by Pauzenga (1985) i.e: ME(kcal/kg) = (37.7 x CP%) + (81.8 x EE%) + (35.5 x NFE%). ME(MJ/kg) = ME(kcal/kg)* (4.18/1000).*

P1: Discarded vegetable, fresh bovine blood and fresh rumen digesta

P2: Discarded vegetable, ensiled bovine blood and fresh rumen digesta

P3: Discarded vegetable, fresh bovine blood and ensiled rumen digesta

Results

Main effect of mixtures from the different processing methods and levels of inclusion on nutrient retention of broiler chickens at starter phase.

The main effect of mixtures from the different processing methods and levels of inclusion in diets on the nutrient retention of starting broilers is shown in Table 3. Crude protein digestibility values ranged from 78.36 to 79.12% with the highest value for the birds fed P3 similar to that of P2 and the least value for the birds fed P1. Birds fed P3 had higher value for ether extract and Nitrogen free extract than birds fed P1 and P2 with lowest values obtained in birds on diet containing P2 and P1 respectively. Crude fibre were not significantly (P>0.05) affected by processed mixtures in diets. The levels of inclusion had no significant (P>0.05) effect on the crude protein, ether extract and fibre.

Details of interaction between mixtures from the different processing methods and levels of inclusion on nutrient retention at starter phase.

The effect of interaction between mixtures from the different processing methods and levels of inclusion on nutrient digestibility

of broiler starter birds shown in Table 4 revealed significant (P<0.05) influence on most parameters except crude digestible fibre. Digestible crude protein was statistically similar (within each processed mixtures at the three levels of inclusion), the values were however highest and similar at 0, 3 and 6 % inclusion in P2 and P3 with lowest values obtained in P1. Birds fed diet containing 0, 3 or 6 % of P1 and P2 had nitrogen-free extract values within similar range. There were significant (P<0.05) differences in the ether extract retention, though; they do not follow a particular pattern with highest values obtained at 3 % inclusion in P3.

The main effect of mixtures from the different processing methods and levels of inclusion on nutrient retention of finishing broilers.

The main effect of processed mixtures on nutrient retention of finishing birds shown in Table 5 revealed significant (P<0.05) influence on ether extract and crude fibre. Digestibility of ether extract and crude fibre were highest in birds fed P3 while lowest value was obtained in birds on diets P1 and P2, respectively.

The levels of inclusion had significant (P<0.05) effect on nitrogen-free extract and crude fibre retention. Digestible nitrogen-free extract obtained at 0 and 3% levels of inclusion were statistically similar (P>0.05) with birds at 6 % level of inclusion having the best value. However, the digestibility of crude fibre decreases with increasing level of inclusion in the diets. There were no significant (P>0.05) differences in digestibility of crude protein and ether extract.

Details of interaction between mixtures from the different processing methods and levels of inclusion on nutrient retention at finisher phase.

The effect of interaction between mixtures from the different processing methods and levels of inclusion is shown in Table 6. The interaction had significant (P<0.05) influence on most parameters except digestibility of crude protein. It was observed that birds fed P1 at 6 % inclusion level recorded the highest digestibility of nitrogen free extract while values obtained in 0, 3 and 6 % levels of inclusion of P3 were similar with others not following a particular trend. However, increasing level of inclusion of the three processed mixtures revealed an increase in digestibility of ether extract of the birds except at P3 while a decline in digestibility of crude fibre in 0, 3 and 6 % inclusion in P1 and P2 only was obtained.

Main effect of mixtures from the different processing methods and levels of inclusion on cost benefit of rearing broiler chickens

The main effect of the mixtures from the different processing methods and levels of inclusion on cost benefit of rearing broiler chickens at starter and finisher phases of growth is presented in Table 7. At the starter phase of growth processed mixtures had no significant (P>0.05) effect on all parameters except total weight gain. Total weight gain was highest in birds fed diet containing P3 and lowest in birds fed P2. It was also however observed that processed mixtures had no effect (P>0.05) on all economic indices at the finisher phase of growth.

Levels of inclusion had no significant (P>0.05) influence on all economic parameters at the starting phase of growth. The economic analysis/benefit however revealed a decrease in cost/kg feed and feed intake of birds as the level of inclusion in diets increased from 0 to 6 %. A similar trend was also observed in Total Feed intake and Total weight gain. The cost of 1kg feed consumed and cost/kg feed were statistically similarly across the level of inclusion, though it revealed that it was cheaper to include at 6% in the diets of birds as the cost of 1kg feed consumed/weight gain gave almost equal values as that obtained at 0% and 3% level of inclusion.

In addition, at the finisher phase of growth, levels of inclusion had effect (P>0.05) Total Feed intake, Total weight gain, FCR and Cost/kg weight gain. The best FCR and Cost/kg weight gain values were obtained in birds at 0% level of inclusion while values for 3 and 6 % level of inclusion were statistically similar.

Table 2: Composition (%) of Broiler finisher diets

	1	2	3	4	5	6	7	8	9
	0%	3%	6%	0%	3%	6%	0%	3%	6%
Ingredient									
Maize	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00
Wheat offal	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Groundnut Cake	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	15.00	12.75	10.50	15.00	12.75	10.50	15.00	12.75	10.50
P 1	-	2.25	4.50	-	-	-	-	-	-
P 2	-	-	-	-	2.25	4.50	-	-	-
P 3	-	-	-	-	-	-	-	2.25	4.50
Fish meal (72%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
*Vit./Min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Palm-Oil	2.50	2.50							
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Determined analysis									
Crude protein (%)	22.57	21.28	21.43	22.57	21.07	21.00	22.57	21.08	21.03
Crude fibre (%)	3.98	4.02	4.05	3.98	3.43	4.28	3.98	4.11	4.23
Ether extract (%)	4.05	5.85	6.40	4.05	4.65	4.92	4.05	5.71	5.71
**ME(MJ/kg)	12.95	10.76	9.51	12.95	11.65	11.61	12.95	10.70	10.51

**Premix composition per kg diet:Vit A :400000IU,Vit D:80000IU,Vit E:40000ng,Vit k3:800mg,Vit B1:1000MG,Vit B2:6000mg, Vit B6:500mg,VitB12:25mg, Niacin:6000mg, Panthothenic acid:2000mg, Folic acid: 200mg, Biotin:8mg, Manganese:300000g, Iron:8000mg, Zinc:20000g, Cobalt:80mg, Iodine:400mg, Selenium:40mg, Choline:800000g*

**Estimated using the formula by Pauzenga (1985) i.e: ME(kcal/kg) = (37.7 x CP%) + (81.8 x EE%) + (35.5 x NFE%). ME(MJ/kg) = ME(kcal/kg)* (4.18/1000).*

P1: Discarded vegetable, fresh bovine blood and fresh rumen digesta

P2: Discarded vegetable, ensiled bovine blood and fresh rumen digesta

P3: Discarded vegetable, fresh bovine blood and ensiled rumen digesta

Table 3: Main effect of mixtures from the different processing methods and levels of inclusion on nutrient retention of broiler chickens at starter phase

	Processed Mixtures				Levels of Inclusion				
Parameter	**P1**	**P2**	**P3**	**SEM**	**0%**	**3%**	**6%**	**SEM**	**P x LI**
Crude protein(%)	78.36^{b}	79.05^{a}	79.12^{a}	0.07	78.87	78.82	78.82	0.14	*
Nitrogen-free extract(%)	75.45^{c}	76.22^{b}	79.03^{a}	0.15	76.95	77.18	76.58	0.58	*
Ether extract(%)	80.16^{b}	78.48^{c}	81.51a	0.20	80.23	80.04	79.87	0.50	*
Crude fibre(%)	65.83	65.36	66.05	0.34	65.48	66.00	65.76	0.33	NS

$^{a, b, c}$: Means in the same row by factor with different superscripts differ significantly(P<0.05)

SEM: Standard Error of Mean.

**: Significant*

NS: Not significant

P1: Discarded vegetable, fresh bovine blood and rumen digesta

P2: Discarded vegetable, ensiled blood and fresh rumen digesta

P3: Discarded vegetable, fresh bovine blood and ensiled rumen digesta

P x LI: Interaction of Processed mixtures and levels of inclusion

Table 4: Details of interaction between mixtures from the different processing methods and levels of inclusion on nutrient digestibility at starter phase

		Processed Mixtures			
Parameter	**Levels of inclusion**	**P1**	**P2**	**P3**	**SEM**
Crude protein(%)	0	78.37^{b}	79.13^{a}	79.13^{a}	0.15
	3	78.40^{b}	78.96^{a}	79.12^{a}	
	6	78.30^{b}	79.05^{a}	79.12^{a}	
Nitrogen-free extract(%)	0	75.41^{c}	76.17^{c}	78.05^{b}	0.36
	3	75.49^{c}	79.27^{c}	79.26^{b}	
	6	75.46^{c}	76.22^{c}	79.79^{a}	
Ether extract(%)	0	80.10^{bc}	79.17^{cd}	81.43^{ab}	0.46
	3	80.18^{bc}	78.06^{d}	81.88^{a}	
	6	80.19^{bc}	78.19^{d}	81.23^{ab}	

$^{a, b, c, d}$: Means in the same row with different superscripts differ significantly(P<0.05)
SEM: Standard Error of Mean.
P1: Discarded vegetable, fresh bovine blood and rumen digesta
P2: Discarded vegetable, ensiled blood and fresh rumen digesta
P3: Discarded vegetable, fresh bovine blood and ensiled rumen digesta

Table 5: The main effect of mixtures from the different processing methods and levels of inclusion on nutrient retention of finishing broilers.

	Processed Mixtures				Levels of Inclusion				
Parameter	**P1**	**P2**	**P3**	**SEM**	**0%**	**3%**	**6%**	**SEM**	**P x LI**
Crude protein(%)	80.56	80.59	80.46	0.18	80.4	80.58	80.63	0.20	NS
Nitrogen-free extract(%)	78.38	78.15	78.51	0.21	78.24b	77.91^{b}	78.90^{a}	0.18	*
Ether extract(%)	78.86^{a}	78.42^{b}	79.08^{a}	0.28	78.82	78.72	78.83	0.31	*
Crude fibre(%)	63.53^{b}	64.14^{a}	64.28^{a}	0.37	65.34^{a}	63.32^{b}	63.29^{b}	0.19	*

$^{a, b, c}$: Means in the same row by factor with different superscripts differ significantly(P<0.05)
SEM: Standard Error of Mean.
**: Significant*
NS: Not significant
P1: Discarded vegetable, fresh bovine blood and rumen digesta
P2: Discarded vegetable, ensiled blood and fresh rumen digesta
P3: Discarded vegetable, fresh bovine blood and ensiled rumen digesta
P x LI: Interaction of Processed mixtures and levels of inclusion

Details of interaction between mixtures from the different processing methods and levels of inclusion on cost benefit of broiler chicken

The details of interaction between processed mixtures and levels of inclusion on cost benefit of broiler chicken is shown in Table 8. Interaction effect revealed significant (P<0.05) differences in total feed intake, total weight gain cost/total feed intake and cost/kg weight gain. There was a decrease in the total weight gain as the level of inclusion in the processed mixtures; P1 and P2 increased while a reverse trend was observed in P3. There was a decrease in the cost/kg weight gain values in P2 and P3 as the inclusion level increased from 0 to 6 %.

At the finisher phase of growth, FCR, Cost/Total feed intake and Cost/kg weight gain were significantly (P>0.05) influenced by interaction of processed mixtures and level of inclusion. The Cost: Benefit revealed an accent

Table 6: Details of interaction between mixtures from the different processing methods and levels of inclusion on nutrient retention at finisher phase

		Processed Mixtures			
Parameter	**Levels of inclusion**	**P1**	**P2**	**P3**	**SEM**
Nitrogen-free extract(%)	0	77.82^{cd}	78.40^{bc}	78.49^{bc}	0.23
	3	77.79^{cd}	77.40^{d}	78.55^{bc}	
	6	79.52a	78.66^{b}	78.50^{bc}	
Ether extract(%)	0	78.16^{d}	78.03^{de}	80.27^{a}	0.24
	3	78.60^{cd}	78.24^{de}	79.32^{bc}	
	6	79.82^{ab}	79.00^{c}	77.66^{e}	
Crude fibre(%)	0	65.12^{a}	65.70^{a}	65.20^{a}	0.24
	3	62.68^{d}	63.65^{bc}	63.62^{bc}	
	6	62.79^{d}	63.06^{cd}	64.04^{b}	

$^{a, b, c, d}$: Means in the same row with different superscripts differ significantly(P<0.05)
SEM: Standard Error of Mean.
P1: Discarded vegetable, fresh bovine blood and rumen digesta
P2: Discarded vegetable, ensiled blood and fresh rumen digesta
P3: Discarded vegetable, fresh bovine blood and ensiled rumen digesta

in the cost/kg weight gain of birds the level of inclusion of P2 and P3 increased from 0 to 6 % while P1 did not follow a particular trend.FCR value was best at 0% level of inclusion while highest value was obtained in birds fed at 3 % level of inclusion of P1. In addition, cost/kg weight gain were statistically similar at 3 and 6 % level of inclusion of the three mixtures with comparable lowest values obtained at 0% level of inclusion of P1, P2 and P3.

Discussion

Crude protein digestibility values ranged from 78.36 to 79.12% with the highest value for birds on P3 and the least value for birds on P1 at the starter phase. This indicating that birds on P3 though having a comparable value with P2 were able to digest the ingredient when fed in diets. Birds on P3 had higher digestibility for ether extract and nitrogen-free extract than birds fed P1 and P2 with lowest values obtained in birds on diets P1 and P2, respectively. Whitehead and Fisher (1975) observed that dietary fat did improve efficiency of feed utilization of poultry diets and the improvement was attributed to the high energy concentration of fats. The increased digestibility of fat recorded may be attributed to the increased ether extract of the resultant diet. The increased digestibility of the various proximate fractions is a pointer to the fact that the birds were able to meet the requirements for other essential nutrients and that the processing method employed in the preparation of the mixtures was adequate.

Birds on diets containing P3 gave the highest body weight gain and nutrient retention values, while those on fed P1 compounded diets gave a lower weight gain and retention values. According to Mcleod (1982) and Ojewola et al. (2005), the proportion of dietary energy obtained from fats versus carbohydrates exert an effect on appetite through a physiological 'appetite control center' responsible to the blood levels of certain nutrients such as glucose and amino acids. Jensen et al. (1970) and Firman et al. (2008) validated these findings that such an effect might involve an increased ability of the birds to convert dietary energy from fat into stored energy, thereby ensuring a greater increase in dietary intake and the nutrient availability of other ingredients.

Processed mixtures had significant effect on digestible ether extract and crude fibre with the highest values obtained in birds fed diet containing P3 at finisher phase. Levels of inclusion had effect on nitrogen-free extract

and crude fibre digestibilities. There was a slight decrease in crude fibre digestibility as the level of inclusion increased from 0 to 6 %. Generally, fibre sources are believed to have deleterious impact on protein and amino acids digestibility (Adeniji and Jimoh, 2007). The present study contradicts the report that when dietary fibre sources contribute a significant amount of dietary protein, the effect on nutrient digestibility is high. The result of the experiment bears no semblance to this. This deviation may be as a result of the structure of the processed mixtures (plant and animal origin) being different from fibre structure of conventional feedstuffs, combination ration and replacement level in feed and the processing method employed in the preparation of the mixture (Ekunseitan *et al.,* 2013) which might be favoured by the ability of the anaerobic organisms in breaking down the fibre components of the mixtures.

The main effect of inclusion levels on cost benefit of birds revealed that the highest value of total weight gain was obtained in birds fed diet containing P3. The cost of 1kg feed consumed and cost per kg weight of bird had values statistically similarly across processed mixtures though it revealed that it was cheaper to feed P3 to birds, cost of 1kg feed consumed/

Table 7: Main effect of mixtures from the different processing methods and Levels of Inclusion on cost benefit

	Processed Mixtures				Levels of Inclusion				
Parameter	**P1**	**P2**	**P3**	**SEM**	**0%**	**3%**	**6%**	**SEM**	**P x LI**
Starter Phase									
Total Feed intake(g)	1409.22	1371.61	1389.45	59.56	1409.22	1391.85	1369.85	58.36	*
Total weight gain(g)	625.85^{ab}	599.50^{b}	639.67^{a}	30.83	632.56	623.79	608.68	34.37	*
FCR	2.18	2.19	2.10	0.10	2.13	2.17	2.17	0.10	NS
Cost/kg feed (N/kg)	90.15	90.15	90.15	1.30	91.65	90.15	88.65	0.00	NS
Cost/total feed intake (N)	127.12	125.30	125.25	5.97	129.15	127.08	121.44	1.66	*
Cost/kg weight gain (N/kg)	196.54	197.25	189.03	8.72	195.29	195.50	192.03	2.81	*
Finisher Phase									
Total Feed intake(g)	3232.48	3294.52	3307.30	129.40	3173.67^{b}	3310.24^{a}	3350.39^{a}	109.98	NS
Total weight gain(g)	1585.03	1663.84	1653.43	96.40	1655.06	1594.54	1652.70	103.51	NS
FCR	2.43	2.41	2.44	0.11	2.33^{b}	2.50^{a}	2.46^{a}	0.08	*
Cost/kg feed (N/kg)	81.64	81.64	81.64	0.97	82.76	81.64	80.51	0.00	NS
Cost/total feed intake (N)	263.77	268.90	269.96	9.13	262.65	270.23	269.74	9.33	*
Cost/kg weight gain (N/kg)	198.23	197.90	200.45	8.43	192.65^{b}	203.91^{a}	200.04^{ab}	6.84	*

^{a,b}: Means in the same row by factor with different superscripts differ significantly($P<0.05$)
SEM: Standard Error of Mean.
P x LI: Processed mixtures by Level of inclusion interaction
**: Significant*
NS: Not significant

Table 8: Details of interaction between mixtures from the different processing methods and levels of inclusion on cost benefit of broiler chickens

Processed Mixtures	**P1**			**P2**			**P3**			
Levels of inclusion	**0%**	**3%**	**6%**	**0%**	**3%**	**6%**	**0%**	**3%**	**6 %**	**SEM**
Parameter										
Starter Phase										
Total Feed intake(g)	1415.77^{ab}	1455.78^{a}	1357.91^{ab}	1430.02^{ab}	1336.57^{b}	1348.25^{ab}	1381.74^{ab}	1383.21^{ab}	1403.40^{ab}	31.81
Total weight gain(g)	662.07^{a}	631.03^{ab}	584.45^{b}	611.55^{ab}	594.76^{b}	592.18^{b}	624.05^{ab}	645.56^{a}	649.41^{a}	15.30
Cost/total feed intake (N)	129.76^{ab}	131.23^{a}	130.16^{ab}	131.06^{a}	125.30^{abc}	119.52^{c}	126.64^{abc}	124.69^{abc}	124.41^{abc}	2.87
Cost/kg weight gain (N/kg)	188.86^{ab}	202.15^{a}	198.58^{ab}	203.23^{a}	196.08^{ab}	192.44^{ab}	193.76^{ab}	188.26^{ab}	185.06^{b}	4.87
Finisher Phase										
FCR	2.32^{b}	2.52^{a}	2.45^{ab}	2.32^{b}	2.47^{ab}	2.44^{ab}	2.34^{b}	2.50^{ab}	2.49^{ab}	0.06
Cost/total feed intake (N)	255.58^{c}	263.23^{ab}	272.51^{ab}	265.84^{bc}	271.26^{ab}	269.59^{ab}	266.54^{bc}	276.21^{a}	267.12^{ab}	5.34
Cost/kg weight gain (N/kg)	191.73^{b}	214.13^{a}	212.76^{a}	192.23^{b}	209.60^{a}	212.47^{a}	193.93^{b}	212.15^{a}	216.24^{a}	4.63

^{a,b}: Means in the same row with different superscripts differ significantly($P<0.05$)
SEM: Standard Error of Mean.

weight gain gave almost equal values. Likewise, there were no differences in FCR of all birds fed diets containing the three test ingredients indicating that the net energy available to the bird was similar (Leeson and Atteh, 1995). This confirms also the fact that animal protein inclusion is a requirement in poultry feed since the blends of the mixtures has the additional advantage of supply minerals, vitamins etc. The varying levels of inclusion in diets of birds (3-6%) reduced the cost/kg weight gain and cost/kg feed but was comparable to the control diets (0% inclusion of each ingredient) at the starter phase. The cost of 1kg feed consumed and cost/kg feed revealed that it was cheaper to include the processed mixtures at 6% in the diets of birds as the cost of 1kg feed consumed/weight gain gave almost equal values as that obtained at 0 and 3% level of inclusion. The cost/kg feed consumed and cost/kg weight gain reduced with increasing level of inclusion of P2 and P3 while the best Total weight gain was obtained at 6 % inclusion of P3. This confirming the ability of the processed ingredient to successfully replace soybean in terms of cost, quality of protein and likewise the overall performance of birds. These values were lower than that obtained at 0% level of inclusion. However, Philip (1984) reported that reducing feed cost was not only to obtain cheaper feed but it was also dependent on the production result obtained this cheaper feed.

The cost: benefit revealed an increase in the cost/kg weight gain of birds when the level of inclusion of P2 and P3 increased from 0 to 6% while P1 did not follow a particular trend at the finisher phase of growth. FCR value was best at 0% level of inclusion while highest value was obtained in birds fed at 3% level of inclusion of P1 and P3. Though this implies that birds consumed more but it resulted in a better weight gain and significantly high value at 3% level of inclusion of P3.

Conclusion

Considering the effects of the factors (processed ingredients and levels of inclusion) on nutrient retention and cost benefits, it is evident that the minimum Cost/kg weight

gain value was obtained in birds at 0% level of inclusion while values for 3 and 6% level of inclusion were statistically similar at the finisher phase. Birds fed diet containing P3 had higher digestibility for crude protein, ether extract and Nitrogen free extract than birds fed P1 and P2 at the starter phase. At the finisher phase, digestibility of ether extract and crude fibre were highest in birds fed P3. Also, digestibility of nitrogen-free extract was best at 6% level of inclusion. Increasing levels of inclusion of the three processed mixtures increased digestibility of ether extract of the birds except at P3.There was a decrease in the cost/kg weight gain values in P2 and P3 as the inclusion level increased from 0 to 6%. Considering the Cost analysis, it is economical to incorporate at 6% inclusion of P3 in the diets of birds as the cost of 1kg feed consumed/weight was lowest.

Impact

The increase in price and production of feed ingredient and feed in developing countries greatly impaired the development of poultry industry. These problems coupled with dearth of quality ingredients can be resolved using this alternative feedstuff capable of being produced using local mechanisms and available animal waste resources.

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References

Adeniji AA. 1996. The value of bovine blood–rumen content meal as a feedstuff for pullets. Ph.D Thesis, University of Ilorin, Ilorin, Nigeria.

Adeniji AA, Jimoh A. 2007. Effects of Replacing Maize with Enzyme - Supplemented Bovine Rumen Content in the Diets of Pullet Chicks. International Journal of Poultry Science 6 (11): 814-817.

Association of Analytical Chemists (AOAC). 2000. Official Methods of Analysis Association of Official Analytical Chemists. 19th edition, Washington DC.

Bourdillon A, Carrie B, Conan L, Duperray J, Huyghebaert G, Leclercq B, Lessire M, McNab J, Wiseman J. 1990. British Poultry Science 31: 557 – 565.

Duncan DB. 1955. Multiple Range Test and Multiple F-test. Biometrics 11: 1-2.

Ekunseitan DA. 2012. Response of broiler chickens to diets containing three differently processed discarded vegetable-bovine blood-rumen content as replacement for soybean meal. M.Agric Thesis, Federal University of Agriculture, Abeokuta, Nigeria.

Ekunseitan DA, Balogun OO, Sogunle OM, Yusuf AO, Ayoola AA, Ekunseitan OF. 2013. Growth response and carcass traits of birds fed diets containing three differently processed discarded vegetable-bovine blood-rumen content mixtures. Bulletin of Animal Health and Production. 61: 75-87.

Ekunseitan DA, Balogun OO, Eruvbetine D, Abiola SS, Sogunle OM, Ogunade IM, Egbeyale LT, Ayoola AA, Akinola OF, Allison IB, Osho SO. 2012. Visual assessment, proximate composition and cost analysis of three differently processed discarded vegetable-bovine blood-rumen content mixtures. Nigerian Journal of Animal Production: 39(2):211-217

Ekunseitan DA, Balogun OO, Sogunle OM, Yusuf AO, Ayoola AA, Egbeyale LT, Adeyemi, OA, Allison IB, Iyanda AI. 2013. Health status of birds fed diets containing three differently processed discarded vegetable-bovine blood-rumen content mixtures. Pakistan Journal of Biological Sciences 16(7):325-331.

Esonu BO, Emanalom OO, Udedibie ABI, Herbert U, Ekpor CF, Okoli IC, Iheukwumere FC. 2001. Performance and blood chemistry of weaner pigs fed raw mucuna (velvet bean) meal. Tropical Animal Production Investigation 4: 49-54.

Fetuga BL, Tewe OO. 1985 Potentials of Agro Industrial By-products and Crop Residues as Animal Feeds, Nig. Food J. 2 (2): 136 –141.

Firma JF, Kamyab A, Leigh H. 2008. Comparison of Fat Sources in Rations of Broilers from Hatch to Market. International Journal of Poultry Science 7 (12): 1152-1155.

Leeson S, Atteh JO. 1995. Utilization of fats and fatty acids by turkey poults. Poultry Science 74: 2003-2010.

Mcleod JA. 1982. Nutritional factors influencing carcass fat in broilers -A review. World's Poultry Science Journal 38 (93): 194-200.

NRC (National Research Council) 1994. .Nutrient Requirements of Domestic Animals. Nutrient Requirements of Poultry, 9th revised ed. National AcademyPress, Washington, DC, USA.

Ojewola GS, Okoye FC, Ukoha OA. 2005. Comparative Utilization of Three Animal Protein Sources by Broiler Chickens. International Journal of Poultry Science 4 (7): 462-467.

Omole TA, Tewe OO. 1991. Processing Technology for Nigerian Feed Stuffs. Document Prepared for the presidential Task Force on Alternative Feed Formulation for Nigeria, pp. 183

Onibi GE, Owoyemi AP, Akinyemi OO. 1999. Diets and dietary ingredients selection by broiler chicken: Effects on growth performance, carcass quality and economics of production. Nig. J. Anim. Prod., 26: 35-42.

Philip GD. 1984. Feed utilization: Principles and new development in physiology. Canadian Journal of Animal Science. 64: 543-549.

Statistical Analysis Systems. 2011. Version 9.1.3, SAS, Institute Inc. Carry N.C USA.

Whitehead CC, Fisher C. 1975. The utilization of various fats by Turkeys of different ages. British Poultry Science, 16: 481 – 485.

THE IMMUNOLOGICAL RELATIONSHIP BETWEEN TYPANOSOMA EVANSI AND *TRYPANOSOMA VIVAX*: IMMUNIZATION BY INFECTION, TREATMENT AND CHALLENGE FINDINGS

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Summary

Trypanosoma evansi and *Trypanosoma vivax* show very high cross-reactivity in serology; and previous serum neutralization studies demonstrated that anti *T. evansi* serum contained lytic antibodies that could lyse a large proportion of *T. vivax* trypomastigotes though anti *T. vivax* serum had no effect on *T. evansi* trypomastigotes. This supports the suspicion that the two parasite species are immunologically closely related and prior infection with one species could cross protect against infection with the other species. The possibility of mice infected with *T. evansi* then treated and challenged with *T. vivax* being protected against the latter parasite infection and vice versa was investigated. Mice were infected with *T. evansi*, then treated with Diminasan after the infection had become patent and challenged with *T. vivax* and vice versa. The challenged mice were monitored for patent infection through examination of their tail blood to establish whether there was any cross protection. Mice were also infected, treated and challenged with homologous trypanosome species to establish whether there was any protection after prior infection followed by treatment and homologous challenge. Mice without prior infection but treated then challenged and those without prior infection and not treated but challenged were included as unexposed and Deminasan controls, respectively. The mean survival time of the Deminasan control mice was not significantly different ($p = 0.246183 > 0.05$) from the mean survival time of the unexposed control mice. However, mice previously exposed to *T. evansi* infection then challenged with *T. vivax* had a significantly higher mean survival time ($p = 0.022055 < 0.05$) compared to the unexposed control mice. Similarly the mean survival times of mice without prior exposure to *T. vivax* infection but treated then challenged with *T. evansi* (unexposed control) and those without prior infection and not treated but challenged (Deminasan control) were not significantly different ($p = 0.122966 > 0.05$). The mean survival time of mice previously exposed to *T. vivax* infection then challenged with *T. evansi* was significantly higher compared to the mean survival times of the unexposed and Deminasan control mice ($p = 0.01622 < 0.05$). Previous exposure to *T. evansi* infection followed by treatment and homologous challenge conferred 50% protection to mice previously infected with *T. evansi* then treated and challenges with *T. evansi*. While previous exposure to *T. vivax* infection followed by treatment and homologous challenge only prolonged the mean survival time of mice previously infected with *T. vivax* then treated and challenged with *T. vivax*.

The above findings, therefore, suggest that *T. evansi* and *T. vivax* are immunologically related and prior infection with one species prolongs the survival time of mice previously infected with one species when challenged with the other. Previous exposure to *T. evansi* infection followed by homologous challenge confers 50% protection to mice previously exposed to *T. evansi* infection. While previous exposure to *T. vivax* infection followed by homologous challenge only prolongs the mean survival time of mice previously exposed to *T. vivax* infection.

Key words: Immunological relationship, *T. evansi*, *T. vivax*, cross protection

LA RELATION IMMUNOLOGIQUE ENTRE TYPANOSOMA EVANSI ET *TRYPANOSOMA VIVAX* : RESULTATS SUR L'IMMUNIZATION PAR INFECTION, TRAITEMENT ET EPREUVE VIRULENTE

Résumé

Les espèces *Trypanosoma evansi* et *Trypanosoma vivax* montrent une très forte réactivité croisée en sérologie ; et des études antérieures de neutralisation de sérum ont démontré que le sérum anti *T. evansi* contenait des anticorps lytiques susceptibles de lyser une grande proportion des trypomastigotes de *T. vivax*, tandis que le sérum anti *T. vivax* n'a eu aucun effet sur les trypomastigotes de *T. evansi*. Ceci confirme la suspicion que les deux espèces de parasites ont un lien étroit entre elles sur le plan immunologique, et qu'une infection antérieure par une espèce peut assurer une protection horizontale contre les infections par les autres espèces. La possibilité de protection des souris infectées par *T. evansi* et ensuite traitées et soumises à une épreuve virulente à *T. vivax* contre cette dernière infection parasitaire et vice versa a été étudiée. Des souris ont été infectées avec *T. evansi*, puis traitées avec du diminasan après la manifestation de l'infection et ensuite soumises à une épreuve virulente à *T. vivax* et vice versa. Les souris soumises à l'épreuve virulente ont été suivies pour détection de l'infection manifeste par examen du sang de leur queue afin de déterminer l'éventualité d'une protection croisée. Les souris ont été également infectées, traitées et soumises à une épreuve virulente avec des espèces de trypanosomes homologues en vue de déterminer la possibilité d'une éventuelle protection, après une infection suivie d'un traitement et d'une épreuve virulente utilisant des espèces homologues. Les souris n'ayant eu aucune infection préalable mais traitées puis soumises à l'épreuve virulente et celles sans infection préalable et non traitées mais soumises à l'épreuve virulente ont été inclues respectivement comme témoins non exposés et sous diminasan. Le temps de survie moyen des souris témoins sous diminasan n'était pas significativement ($p = 0,246183 > 0,05$) différent du temps moyen de survie des souris témoins non exposées. Cependant, les souris préalablement exposées à l'infection à *T. evansi* et ensuite exposées à *T. vivax* avaient un temps de survie moyen nettement plus élevé ($p = 0,022055 < 0,05$) par rapport aux souris témoins non exposés. De même, le temps de survie moyen des souris non préalablement exposées à l'infection à *T. vivax* mais traitées puis soumises à une épreuve virulente de *T. evansi* (témoin non exposé) et celles non préalablement infectées et non traitées mais soumises à une épreuve virulente (témoins sous diminasan) n'étaient pas significativement ($p = 0,122966 > 0,05$) différents. Le temps de survie moyen des souris préalablement exposées à l'infection *T. vivax* et ensuite soumises à l'épreuve virulente de *T. evansi* était significativement plus élevé par rapport au temps de survie moyen des souris témoins non exposées et celles sous diminasan ($p = 0,01622 < 0,05$). Une exposition antérieure à l'infection *T. evansi* suivie d'un traitement et d'une épreuve virulente avec les espèces homologues a conféré une protection de 50% aux souris préalablement infectées avec *T. evansi* et ensuite traitées et soumises à une épreuve virulente à *T. evansi*. Une exposition antérieure à l'infection à *T. vivax* suivie d'un traitement et d'une épreuve virulente d'espèces homologues a seulement prolongé le temps de survie moyen des souris préalablement infectées avec *T. vivax* puis traitées et exposées à *T. vivax*.

Les résultats ci-dessus portent à croire que *T. evansi* et *T. vivax* sont immunologiquement apparentés et qu'une infection antérieure par une espèce prolonge le temps de survie des souris préalablement infectées par une espèce en cas d'exposition à l'autre espèce. Une exposition antérieure à l'infection *T. evansi* suivie d'une épreuve virulente homologue confère une protection de 50% aux souris préalablement exposées à l'infection à *T. evansi*. L'exposition antérieure à l'infection à *T. vivax* suivie d'une épreuve virulente homologue ne fait que prolonger le temps de survie moyen des souris préalablement exposées à l'infection à *T. vivax*.

Mots-clés : relation immunologique, *T. evansi*, *T. vivax*, protection croisée

Introduction

Trypanosomes causing disease in livestock include Trypanosoma congolense, Trypanosoma simiae, Trypanosoma brucei brucei, *Trypanosoma vivax*, *Trypanosoma evansi* and Trypanosoma equiperdum; let alone Trypanosoma brucei gambiense, Trypanosoma brucei rhodesiense and Trypanosoma cruzi species which cause disease in humans (1). Among the species causing disease in livestock, *T. evansi* and *T. vivax* are the most widely

distributed in the world, occurring in tropical Africa (1), south eastern Asia (2) Middle East and South America (3, 4 and 5). The two parasite species pose a disease threat to 500 million cattle, 100 million domestic buffaloes and 12 million camels, horses and other domestic animal species found in parts of the world where the disease is endemic (6). *T. evansi* and *T. vivax* antigens show high immunological cross reactivity in capillary agglutination, indirect enzyme linked immunosorbent assay (ELISA), western immunoblots and immunofluorescence microscopy (7). *T. evansi* antigens have also been used to detect antibodies produced in response to *T. vivax* infection (8, 9 and10). This suggests that *T. evansi* and *T. vivax* are immunologically related and previous serum neutralization studies demonstrated that anti *T. evansi* serum contained lytic antibodies that could lyse a large proportion of *T. vivax* trypomastigotes though anti *T. vivax* serum had no effect on *T. evansi* trypomastigotes (11). In 1986 Olaho-Mukani observed that camels previously exposed to *T. evansi* infection and deliberately exposed to tsetse challenge for one year on Galana Ranch, Kenya, where *T. vivax* infection was rampant in cattle, failed to contract the latter infection, but succumbed to T. congolense (pers. com.). Yet, camels are susceptible to *T. vivax* (12). This supports the suspicion that the two infections probably do cross-protect against each other, a possibility that had never been investigated. Uzcanga et al. (2002) identified some antigens responsible for the serological cross reactivity between *T. evansi* and *T. vivax* by immunobloting, and found a series of polypeptide species ranging from 14 – 109kDa in the clarified soluble antigenic fraction of *T. evansi* to be common antigens for both anti *T. evansi* equine antibodies and anti *T. vivax* bovine antibodies. Among the identified *T. evansi* cross-reacting antigens, a 64kDa antigen was purified and immunofluorescence microscopy indicated the latter to be primarily localized on the parasite surface (10). Protective antibodies are surface specific and the host response to the parasite surface antigens plays an important role in controlling the parasite infection (13). Trypanosomes change their surface antigens through variation of surface glycoprotein antigens (14). During each peak parasitaemia, a mixture of variable antigenic types of parasites is present but the dominant antigenic type parasite population determines the specific antibody response. The specific antibodies produced in response to the infection kill off the dominant antigenic parasite population, leaving a small non-dominant antigenic parasite population to multiply and the process continues in cycles until the animal dies if not treated or the immune mechanisms catch up with the parasite and the animal recovers on its own (15). This phenomenon is responsible for the successive waves of parasitaemia in infected animals. There is also evidence that following repeated episodes of infection and recovery (with or without treatment) in an enzootic area, animals encounter a variety of antigenic types and become less susceptible to strains in that area (14). African trypanosomes also possess protein fractions which if used to immunize small laboratory mammals or large animals; they protect or partially protect the immunized animals or the animals can live longer than the controls upon homologous challenge (16; 17; 18; 19; 20 and 21). Parasite antigens continue to circulate in the host's body for some time, for as long as six months even after the parasites have been cleared by treatment (22). And the infection and treatment method has been reported to increase the prepatent period and survival time after challenge but does not prevent infection (23).

The purpose of this study was to establish whether infection with one trypanosome species could cross protect against infection with the other species before embarking on identification, isolation, characterization and determination of the immunological properties of the two Trypanosoma species proteins for possible use in serodignosis and vaccine production.

Materials and Methods

Cloned trypanosome stocks were obtained from Kenya Trypanosomiasis Research Institute (KETRI), Muguga and Faculty of Veterinary Medicine Makerere University, Kampala. They included *T. evansi* (KETRI 2540),

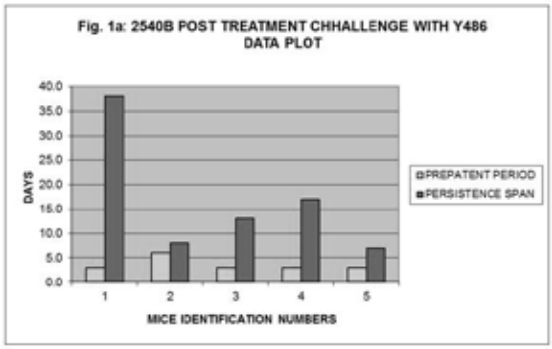

Figure 1a: Pre-patent periods and survival time of mice immunized by infection with *T. evansi* then treated (Group 2540B) and challenged with *T. vivax* (Y486).

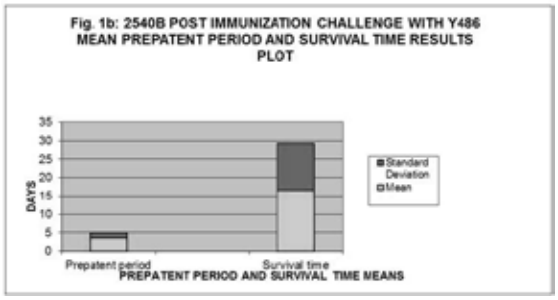

Figure 1b: Mean pre-patent period and survival time of mice immunized by infection with *T. evansi* then treated (Group 2540B) and challenged with *T. vivax* (Y486).

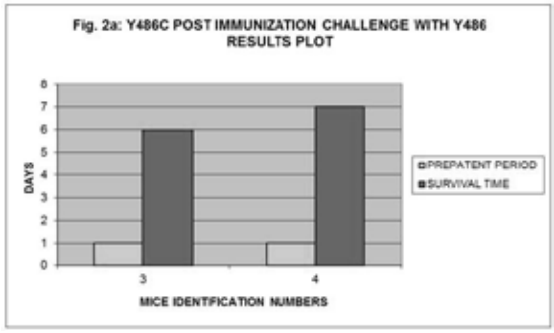

Figure 2a: Pre-patent periods and survival time of mice not previously infected with *T. evansi* but treated and then challenged with *T. vivax* (*T. vivax* control or Y486C).

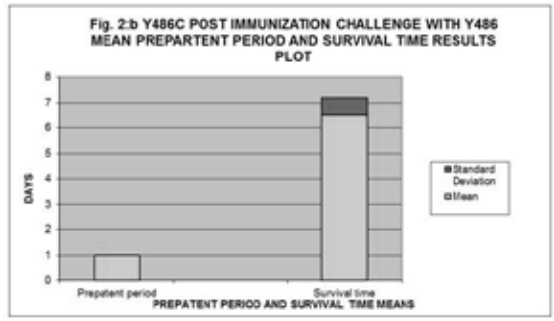

Figure 2b: Mean pre-patent period and survival time of mice not previously infected with *T. evansi* but treated and then challenged with *T. vivax* (*T. vivax* control or Y486C).

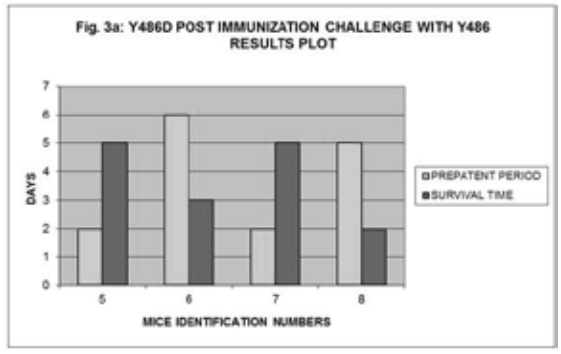

Figure 3a: Pre-patent periods and survival time of mice not previously infected with *T. evansi* and not treated but challenged with *T. vivax* (*T. vivax* diminasan control or Y486D).

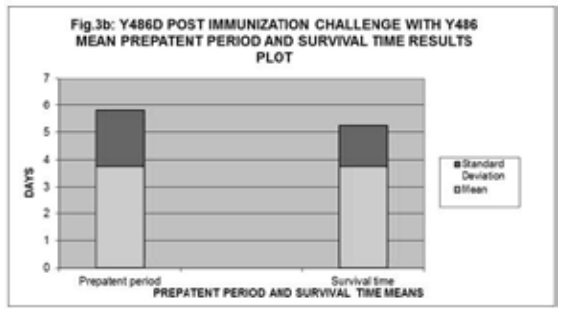

Figure 3b: Mean pre-patent period and survival time of mice not previously infected with *T. evansi* and not treated but challenged with *T. vivax* (*T. vivax* diminasan control or Y486D).

Table 1: Patent rates, mean pre-patent periods, protection rates and mean survival times of mice immunized by infection with *T. evansi* then treated and challenged with *T. vivax* (2540B), mice without prior immunization by infection with *T. evansi* but treated and challenged with *T. vivax* (Y486C) and mice without prior immunization by infection with *T. evansi* and treatment but challenged with *T. vivax* (Y486D). Y486C and Y486D were *T. vivax* and Diminasan controls respectively.

Mice group	Patent rate	Mean pre-patent period	Protection rate	Mean survival time
2540B	100%	3.6	0%	16.6
Y486C	100%	1.0	0%	6.5
Y486D	100%	3.8	0%	3.8

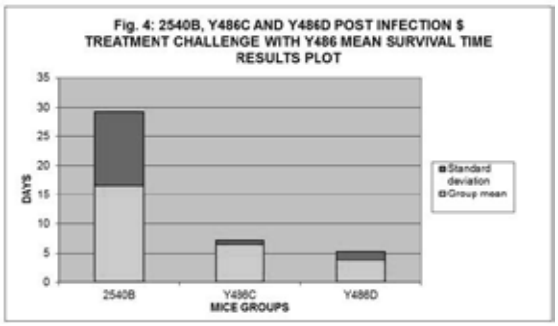

Figure 4: Mean survival time of mice immunized by infection with *T. evansi* then treated and challenged with *T. vivax* (Group 2540B); mice without prior immunization by infection with *T. evansi* but treated and challenged with *T. vivax* (Group Y486C) and mice without prior immunization by infection with *T. evansi* and treatment but challenged with *T. vivax* (Group Y486D). Y486C and Y486D were *T. vivax* and Diminasan controls, respectively.

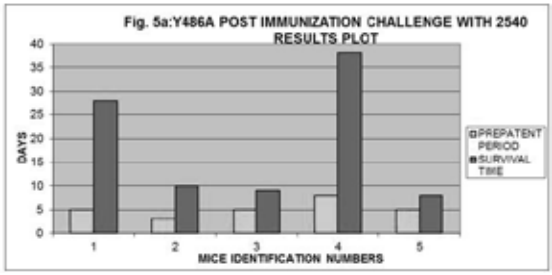

Figure 5a: Pre-patent periods and survival time of mice immunized by infection with *T. vivax* then treated and challenged with *T. evansi* (Group Y486A).

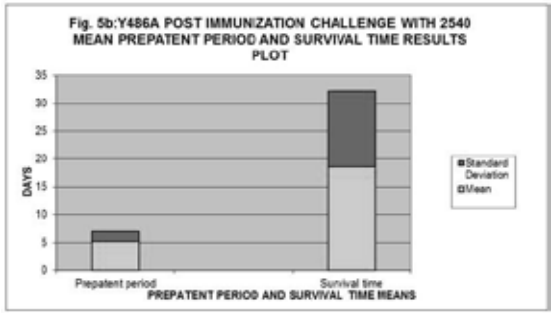

Figure 5b: Mean pre-patent period and survival time of mice immunized by infection with *T. vivax* then treated and challenged with *T. evansi* (Group Y486A).

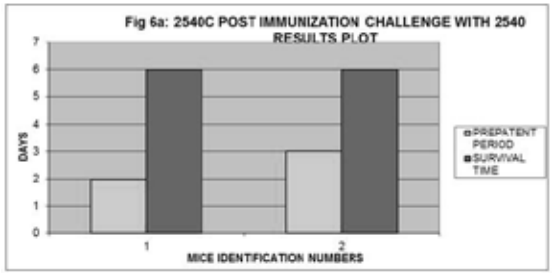

Figure 6a: Pre-patent periods and survival time of mice previously not immunized by infection with *T. vivax* but treated and challenged with *T. evansi* (2540 control mice or 254^{0}C).

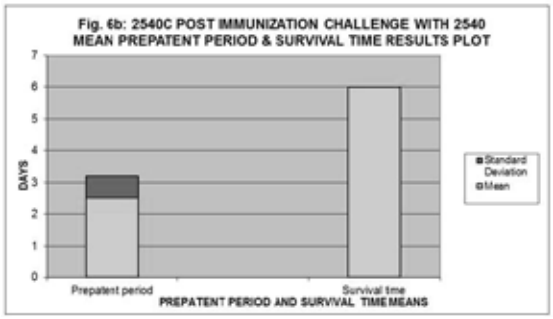

Figure 6b: Mean pre-patent periods and survival time of mice previously not immunized by infection with *T. vivax* but treated and challenged with *T. evansi* (2540 control mice or 254^{0}C).

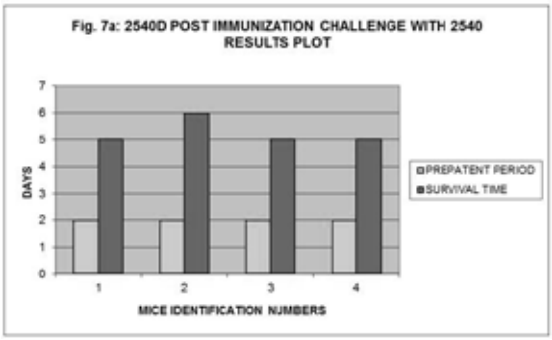

Figure 7a: Pre-patent periods and survival time of mice previously not immunized by infection with *T. vivax* and not treated but challenged with *T. evansi* (2540 Diminasan control mice or 2540D).

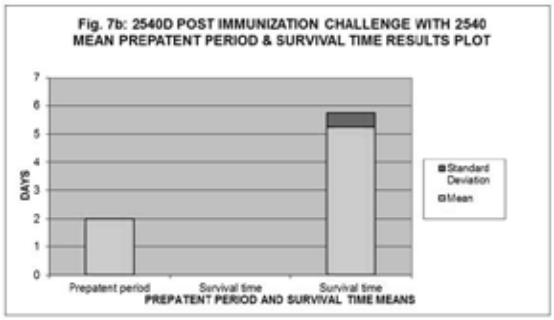

Figure 7b: Mean pre-patent periods and survival time of mice previously not immunized by infection with *T. vivax* and not treated but challenged with *T. evansi* (2540 Diminasan control mice or 2540D).

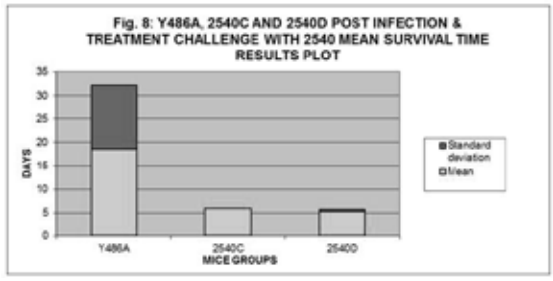

Figure 8: Mean survival time of mice immunized by infection with *T. vivax* then treated and challenged with *T. evansi* (Group Y486A); mice without prior immunization by infection with *T. vivax* but treated and challenged with *T. evansi* (Group 2540C) and mice without prior immunization by infection with *T. vivax* and treatment but challenged with *T. evansi* (Group 2540D). 2540C and 2540D were *T. evansi* and Diminasan controls, respectively

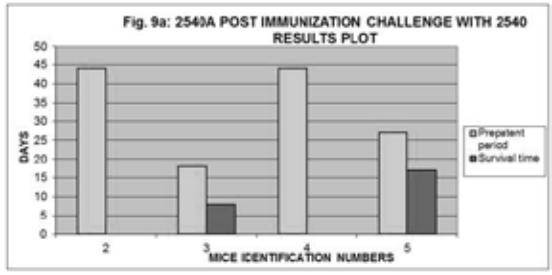

Figure 9a: Pre-patent periods and survival time of mice immunized by infection with *T. evansi* then treated (Group 2540A) and challenged with *T. evansi* (2540).

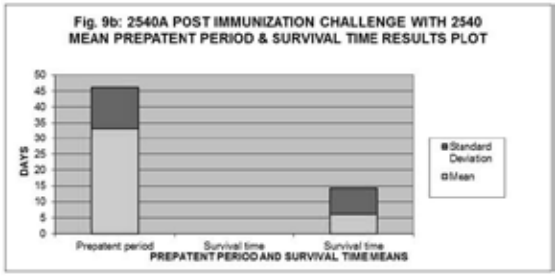

Figure 9b: Mean pre-patent period and survival time of mice immunized by infection with *T. evansi* then treated (Group 2540A) and challenged with *T. evansi* (2540).

Table 2: Shows patent period, mean pre-patent periods, protection rates and mean survival time of mice immunized by infection with *T. vivax* then treated and challenged with *T. evansi* (Y486A), mice without prior immunization by infection with *T. vivax* but treated and challenged with *T. evansi* (2540C) and mice without prior immunization by infection with *T. evansi* and treatment but challenged with *T. vivax* (2540D). 2540C and 2540D were *T. evansi* and Diminasan controls respectively.

Mice group	Patent rate	Mean pre-patent period	Protection rate	Mean survival time
Y486A	100%	5.2	0%	18.6
2540C	100%	2.5	0%	6.0
2540D	100%	2.0	0%	5.3

from Muguga, Kenya and mouse adapted *T. vivax* (Y486), from the Faculty of Veterinary Medicine, Makerere University, Kampala. KETRI 2540 is said to have originated from Carimagua, Columbia S. America and Y486 from S. Africa. They were stored at-196oC under liquid Nitrogen until required.

Mice were infected with *T. evansi* stock, and then treated once with Diminasan intraperitoneally at a dose rate of 157 mg per 10 Kg body weight (equivalent to 0.157 mg per 10 gm body weight) after patent infection had been detected through examination of tail blood and challenged with *T. vivax* stock and vice versa. After which the mice were monitored for infection to establish whether there was any cross protection. Mice without prior infection but treated then challenged and those without prior infection and not treated but challenged were included as unexposed and Deminasan controls, respectively. Infection, treatment and challenge with homologous trypanosome stocks possible outcomes were also investigated to establish whether homologous challenge confers any protection.

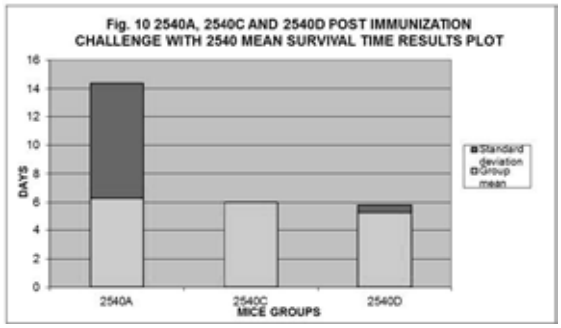

Figure 10: Mean survival time of mice immunized by infection with *T. evansi* then treated and challenged with *T. evansi* (Group 2540A); mice without prior immunization by infection with *T. evansi* but treated then challenged with *T. evansi* (Group 2540C) and mice without prior infection with *T. evansi* and treatment but challenged with *T. evansi* (Group 2540D). 2540C and 2540D were *T. evansi* and Diminasan controls, respectively.

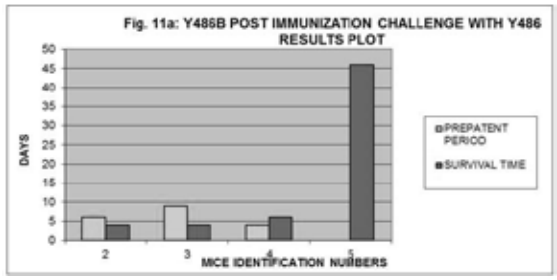

Figure 11b: Mean pre-patent period and survival time of mice immunized by infection with *T. vivax* then treated (Group Y486B) and challenged with *T. vivax* (Y486).

Results

Immunization by infection and treatment results for T. evansi prior infection and post immunization challenge with T. vivax

The patent rate, mean pre-patent period, protection rate and mean survival time were 100%, 3.6 ± 1.341641 (Standard deviation) days, 0% and 16.6 ± 12.62141 (Standard deviation) days, respectively in mice previously infected with *T. evansi* (2540B) then treated and challenged with *T. vivax* (Y486) (Figures 1a, 1b and Table 1). The patent rate, mean pre-patent period, protection rate and mean survival time in mice that were not previously infected with *T. evansi* but treated then challenged with Y486 (*T. vivax* unexposed control or Y486C) were 100%, 1 ± 0 (Standard deviation) day, 0% and 6.5 ± 0.707107 (Standard deviation) days, respectively (Figures 2a, 2b and Table 1), while in mice that were not previously infected with

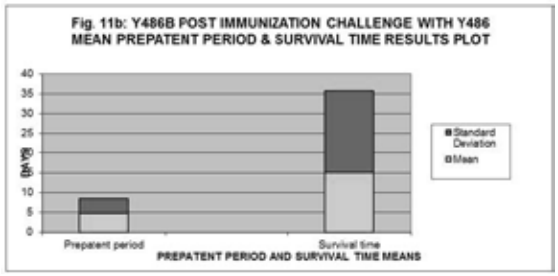

Figure 12: Mean survival time of mice immunized by infection with *T. vivax* then treated and challenged with *T. vivax* (Group Y486B); mice without prior immunization by infection with *T. vivax* but treated then challenged with *T. vivax* (Group Y486C) and mice without prior immunization by infection with *T. vivax* and treatment but challenged with *T. vivax* (Group Y486D). Y486C and Y486D were *T. evansi* and Diminasan controls respectively.

T. evansi and not treated but challenged with *T. vivax* (Diminasan control or Y486D) were 100%, 3.8 ± 2.061553 (Standard deviation) days, 0% and 3.8 ± 1.5 (Standard deviation) days respectively (Figures 3a, 3b and Table1). The results thus show that the mean survival time of Y486D mice was not significantly different ($p = 0.246183 > 0.05$) from the mean survival time of Y486C mice (Fig. 4). However, 2540B mice had a significantly higher mean survival time ($p = 0.022055 < 0.05$) compared to the *T. vivax* unexposed control (Y486C) mice (Fig. 4).

***Immunization by infection and treatment results for T. vivax prior infection followed by post immunization challenge with* T. evansi**

The patent rate, mean pre-patent period, protection rate and the mean survival time were 100%, 5.2 ± 1.788854 (Standard deviation) days, 0% and 18.6 ± 13.63085 (Standard deviation) days respectively in mice previously infected with *T. vivax* then treated and challenged with *T. evansi* (Y486A) (Figures 5a, 5b and Table 2). The patent rate, mean pre-patent period, protection rate and the mean survival time for mice previously not infected with *T. vivax* but treated and challenged with *T. evansi* (2540 unexposed control mice or 2540C) were 100%, 2.5 ± 0.707107 (Standard deviation) days, 0% and 6.0 ± 0 (Standard deviation) days respectively (Figures 6a, 6b and Table 2). While the patent rate, mean pre-patent period, protection rate and the mean survival time for mice not previously infected with *T. vivax* and not treated but challenged with *T. evansi* (2540 Diminasan control mice or 2540D) were 100%, 2.0 ± 0 (Standard deviation) days, 0% and 5.3 ± 0.5 (Standard deviation) days respectively (Figures 7a, 7b and Table 2). The results thus show that the mean survival time of 2540D mice was not significantly different ($p = 0.122966 > 0.05$) from the mean survival time of 2540C mice (Fig. 8). However, Y486A mice had a significantly higher mean survival time compared to the unexposed control (2540C) mice ($p = 0.01622 < 0.05$) (Fig. 8).

Immunization by infection and treatment results for T. evansi and post immunization challenge with T. evansi

Patent rate of 50%; mean pre-patent period of 33.3 days; protection rate of 50% and mean survival time of 6.3 ± 8.098354 (Standard deviation) days were apparent in mice previously infected with *T. evansi* then treated and challenged with *T. evansi* (Group 2540A). The mean survival time for unexposed control mice (2540C) and Diminasan control mice (2540D) were found to be 6.0 ± 0 (Standard deviation) and 5.3 ± 0.5 (Standard deviation) days respectively. This shows that although 2540A mice had an apparently higher mean survival time compared to the unexposed control (254^0C) mice but the time was not significantly different ($p = 0.48457 > 0.05$) from that of 254^0C mice (Figs. 9a, 9b and 10).

Immunization by infection and treatment results for T. vivax followed by post immunization challenge with T. vivax

The patent rate of 100%, mean pre-patent period of 4.8 ± 3.774917 (Standard deviation) days, protection rate of 0% and mean survival time of 15 ± 20.68816 (Standard deviation) days were observed in mice previously infected with *T. vivax* then treated and challenged with *T. vivax* (Group Y486B). The mean survival time for unexposed control mice (Y486C) and Diminasan control mice (Y486D) were 6.5 ± 0.707107 (Standard deviation) and 3.8 ± 1.5 (Standard deviation) days respectively

(as already stated above). This indicates that the Y486B mice mean survival time was apparently higher compared to that of Y486C mice but not significantly different ($p = 0.306509 > 0.05$) from that of the latter mice group (Figs. 2b, 3b, 11a, 11b and 12).

Discussion

Trypanosomes change their surface antigens through variation of surface glycoprotein antigens (14). During each peak parasitaemia, a mixture of variable antigenic types of parasites is present but the dominant antigens determine the specific antibody response. The specific antibodies produced in response to the infection eliminate the dominant antigenic parasite population, leaving a small non-dominant antigenic parasite population to multiply and the process continues in cycles until the animal dies if not treated or the immune mechanisms catch up with the parasite and the animal recovers on its own (15). Repeated episodes of infection and recovery (with or without treatment) confers less susceptibility to trypanosome infection (14) and infection, treatment followed by challenge method of immunization increases the mean survival time of the immunized animals but does not prevent infection (23). In 1986, Olaho-Mukani observed that camels previously exposed to *T. evansi* infection and deliberately exposed to tsetse challenge for one year on Galana Ranch, Kenya, where *T. vivax* infection was rampant in cattle, failed to contract the latter infection, but succumbed to T. congolense (pers. com.). Yet, camels are susceptible to *T. vivax* (12). And previous serum neutralization studies demonstrated that anti *T. evansi* serum contained lytic antibodies that could lyse a large proportion of *T. vivax* trypomastigotes though anti *T. vivax* serum had no effect on *T. evansi* trypomastigotes (11). African trypanosomes also possess protein fractions which if used to immunize small laboratory mammals or large animals; they protect or partially protect the immunized animals or the animals can live longer than the controls upon homologous challenge (16; 17; 18; 19; 20 and 21).

The observation in this study that mice immunized by infection with *T. evansi* then treated and challenged with *T. vivax* succumbed to infection but had a significantly higher mean survival time ($p = 0.022055 < 0.05$) compared to control mice, suggests that prior exposure to *T. evansi* infection prolongs the survival time of the exposed mice but does not prevent infection when challenged with *T. vivax*. This is in agreement with Scott *et al.,* 1978 observation that infection, treatment followed by challenge method of immunization increases the mean survival time of the immunized animals but does not prevent infection. This partial cross protection partly supports the suspicion that prior exposure to *T. evansi* infection cross protects against *T. vivax* infection and the 1986 Olaho-Mukani observation that camels previously exposed to *T. evansi* infection and deliberately exposed to tsetse challenge for one year on Galana Ranch, Kenya, where *T. vivax* infection was rampant in cattle, failed to contract the latter infection, but succumbed to T. congolense (pers. com.), yet, camels are susceptible to *T. vivax* (12). It also partly supports the observation in previous serum neutralization studies (11) that antibodies produced by the host against *T. evansi* can lyse a substantial number of *T. vivax* trypomastigotes and the suspicion that such antibodies are responsible for the prolonged survival time of mice previously infected with *T. evansi* and treated then challenged with *T. vivax* as observed in this study.

Findings of immunization by infection with *T. vivax* followed by treatment and challenge with *T. evansi* were similar to the outcome of immunization by infection and treatment with *T. evansi* followed by challenge with *T. vivax*. This suggests that *T. vivax* also has protein fractions that can make mice previously exposed to *T. vivax* less susceptible to *T. evansi* infection. However, this finding appears not to be in support of one of the findings of the previous serum neutralization studies which indicated that *T. vivax* antiserum contained no lytic antibodies against *T. evansi* (11). The possible explanation for this disparity is that without medical intervention, as was the case in previous serum neutralization studies (11), *T. vivax* tended to run an acute short course in

the experimental animals used to produce the *T. vivax* antiserum, giving the animals no ample time to produce lytic antibodies against *T. evansi*. It is probable that immunization by infection followed by treatment and challenge gives the experimental animals ample time to produce lytic antibodies against *T. evansi*; since parasite antigens continue to circulate in the host's body for sometime, for as long as six months even after the parasites have been cleared by treatment (22).

The DiminasanR (Diminazene diaceturate and antipyrine) controls showing no prolonged prepatent period compared to other mice groups in this particular experiment suggests that the drug had no residual effect that could have interfered with the outcomes of this study.

The observed 50% protection rate in mice with previous exposure to *T. evansi* and treated then followed by homologous challenge and the prolonged mean life time observed in mice with prior exposure to *T. vivax* infection then treated and challenged with *T. vivax* but not in the control mice are in support of the existing evidence that repeated episodes of homologous infection and recovery (with or without treatment) confers less susceptibility to trypanosome infection (13) and that African trypanosomes possess protein fractions which if used to immunize small laboratory mammals or large animals; they protect or partially protect the animals or the animals can live longer than the controls upon homologous challenge (16; 17; 18; 19; 20 and 21).

The mean survival time of mice previously infected with *T. vivax* then treated and challenged with *T. vivax* was apparently higher compared to that of control mice, but was not significantly different from that of the latter group of mice. The biological observations in this experiment appear to agree with the results for immunization with *T. evansi* and homologous challenge. They are also in support of the earlier observations by 16; 17; 18; 19; 20 and 21 that previous exposure to trypanosome infection followed by homologous trypanosome challenge makes previously exposed animals live longer than animals without previous exposure to the homologous challenge. Only that, a statistical type II error (that is accepting a false null hypothesis) could have been committed in accepting the null hypothesis that there was no difference between the mean survival periods of the previously exposed mice and that of the control mice.

Conclusion

Previous serum neutralization studies and the current study findings suggest that *T. evansi* and *T. vivax* are immunologically related and prior infection with one species prolongs the survival time of mice previously infected with one species when challenged with the other. Previous exposure to *T. evansi* infection followed by homologous challenge confers 50% protection to mice previously exposed to *T. evansi* infection. While previous exposure to *T. vivax* infection followed by homologous challenge only prolongs the mean survival time of mice previously exposed to *T. vivax* infection. Early correct diagnosis and appropriate medical intervention confers less susceptibility to subsequent homologous infection with either species. And therefore there is need to carry out further investigations to identify, isolate and characterize antigenic protective proteins suggested to be present in T. envansi for possible use as vaccine against *T. vivax* infection. And any antigenic proteins identified as unique to either Trypanosoma species during such investigations can be adopted as candidates for possible use in development of species-specific serological tests since in clinical practice the drugs commonly used to treat trypanosomiasis in animals often do not successfully treat T. envansi infections.

Acknowledgements

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References

Mulligan, W. H. C. (1970). The African trypanosomiases. 1st Ed. 4, 67-68.

Boyd, R. and Mleche, W. C. H. (1985). Isoenzyme analysis of stocks of trypanosomes isolated from cattle in Indonesia. Research in Veterinary Science 39, 388-389.

Shaw, J. and Lainson, R. (1972). Tryoanosoma vivax in Brazil. Annals of Tropical Medicine and Parasitology 66, 25-32.

Wells, E. A. (1984). Animal trypanosomiasis in South America. Preventive Veterinary Medicine 2, 31-41.

Applewhaite, L. M. (1990). Small ruminant trypanosomiasis in Guyana: A preliminary report. British Veterinary Journal 146, 93-94.

Peregrine, A. S. (1994). Chemotherapy and delivery systems; haemoparasites. Veterinary Parasitology 54, 223-248.

Toro, M., Leon, E., Lopez, R. and Ruiza, A. (1980). Resultados de un muestreo sobre tripanomiasis bovina mediante tecnicas serologicas. Veterinaria Tropical 5, 43-50.

Aray, C., Uzcang, G., Soto, H. and Mendoza, M. (1998). Ensayo immunoenzimatico para el diagnostico de la tripanosomiasis bovina causada por Trypanosoma sp. sero prevalencia en el municipio Monagas del Estado Gaurico-Venezuela. Revista Cientitifica, Universidad del Zulia 8, 114-116.

Reyna-Bello, A., Garcia, F., Rivera, M., Sanso, B. and Aso, P. (1998). Enzyme-linked immunosorbent assay (ELISA) for detection of anti-*Trypanosoma evansi* equine antibodies. Veterinary Parasitology 1483 1-9.

Uzcang, G., Mendoza, M., Aso, P. M. and Bubis, J. B. (2002). Purification of a 64kDa antigen from *Trypanosoma evansi* that exhibits cross-reactivity with *Trypanosoma vivax*. Parasitology (2002) 124, 287-299.

Kakaire N M Olaho M W and Lubega G W (2013). The immunological relationship between Typanosoma evansi and *Trypanosoma vivax*: serum neutralization studies findings. Bull. Anim. Hlth. Prod. Afr., (2013), 61, 651-656

Hornby, H. E. (1952). Animal trypanosomiasis in East Africa, 1949. London, HMSO.

Uche, V. E., Ross, C. A. and Jones, T. W. (1992). Identification of the surface components of *Trypanosoma evansi*. Research in Veterinary Science 53, 252-253.

Radostits, O. M., Blood, D. C., and Gay, C. C., Eds (1994). Diseases caused by trypanosomes: In Veterinary Medicine, A textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses 8th Edition. 1212-1222. Bailliere Tindal Publishers 24-28 Oval Road, London NW1 7DX.

Barry, J. D., and Turner, C. M. R. (1991). Parasitology Today 7, 287.

Olenick, J. G., Wolff, R., Nauman, R. K. and J. McLaughin, J. (1988). A flagellar pocket membrane fraction from Trypanosoma brucei rhodesiense: immunogold localisation and non-variant immunoprotection. Infection and Immunity 56 (1988), pp. 92–98.

Powell, C. (1993). Experimental immunity against trypanosomiasis in rabbits. Vaccine 11 (1993), pp. 307–310.

Balaban, N., Waithaka, H. K., Njogu, A. R. and Goldman, R. (1995). Intracellular antigens (microtubule-associated protein copurified with glycosomal enzymes)—possible vaccines against trypanosomiasis. Journal of Infectious Diseases 172 (1995), pp. 845–850.

Mkunza, M., Olaho, W. M. and C. N. Powell, C. N. (1995). Partial Protection against natural trypanosomiasis after vaccination with a flagellar pocket antigen from Trypanosoma brucei rhodesience. Vaccine 13 (1995), pp. 151–154.

ILRI (1998). International Livestock Research Institute. ILRI 1998 Annual Report of the International Livestock Research Institute, ILRI, Nairobi, Kenya.

Lubega, G. W., Byarugaba D. K .and Prichard, R. K. (2002a). Immunization with a tubulin-rich preparation from Trypanosoma brucei confers broad protection against African trypanosomosis. Experimental Parasitology 102, 9-22.

Luckins, A.G. 1986. Antigens and antibodies in fungal and parasitic diseases. Trypanosoma brucei. In R.F. Masseyef, ed. Methods of enzymatic analysis, Volume 11, Antigens and antibodies, p. 351-367. Weinheim, VCH Verlagsgesellschaft mbH.

Scott, J. M., Holmes, P.H., Pays, T., Jennings, F. W. and Urquhart, G. M. (1978). Attempted protection of Zebu cattle against trypanosomiasis using multi-stabilate vaccine. Reviews in Veterinary Science 25 (1978), pp. 115–117.

EFFECT OF *TETRACERA POTATORIA* ON GROWTH AND NON SPECIFIC IMMUNE RESPONSE IN *CLARIAS GARIEPINUS*

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Abstract

A 56 day study was conducted to evaluate the effect of dietary inclusion of *Tetracera potatoria* root extract (TP) on growth and haematological parameters of African catfish, *Clarias gariepinus*. One hundred and twenty juvenile with initial weight of 30 ± 0.3 g were randomly distributed into plastic tanks (45 L) at 10 fish /tank and replicated twice. Experimental animals were fed with commercial fish feed (42% crude protein) at 5% body weight twice a day. Group 1 animals served as control without inclusion of extract, groups 2 and 3 had 250 mg/kg and 500 mg/kg inclusion of TP respectively and group 4 animals had 600 mg/kg inclusion of vitamin C. The parameters were measured were weight, PCV, Hb, RBC WBC, differential WBC count, and weight of viscerals. The mean weight gain (54.5g/fish), final standard length and the viscerotropic index were significantly highest in fish supplemented with 250 mg/kg TP. There was significant change in the blood parameters of treated fish compared with control. It can be concluded that *Tetracera potatoria* had no toxic effects on the fish but they can serve as natural growth promoters in aquaculture production hence lead to increased productivity.

Keywords: *Clarias gariepinus*, growth, haematology, *Tetracera potatoria*

EFFET DES EXTRAITS DE *TETRACERA POTATORIA* SUR LA CROISSANCE ET LA RÉPONSE IMMUNITAIRE NON SPÉCIFIQUE DU POISSON-CHAT AFRICAIN *CLARIAS GARIEPINUS*

Résumé

Une étude de 56 jours a été réalisée dans le but d'évaluer l'effet de l'inclusion d'extraits de racines de *Tetracera potatoria* (TP) dans les aliments sur la croissance et les paramètres hématologiques du poisson-chat africain, *Clarias gariepinus*. Cent vingt juvéniles d'un poids initial de 30 ± 0,3 g ont été répartis de manière aléatoire dans des cuves en plastique (45 L) à raison de 10 poissons / cuve, avec deux répétitions. Les poissons utilisés dans cette expérience ont été nourris avec des aliments industriels pour poissons (42% de protéines brutes) à raison de 5% du poids corporel deux fois par jour. Les poissons du Groupe 1 ont servi de témoins avec une alimentation ne contenant pas d'extraits ; les régimes des Groupes 2 et 3 ont reçu respectivement un supplément de 250 mg / kg et 500 mg / kg de TP ; et les poissons du Groupe 4 ont reçu un régime avec une inclusion de 600 mg / kg de vitamine C dans leurs aliments. Les paramètres mesurés étaient le poids, l'hématocrite, l'hémoglobine, la numération différentielle de globules rouges, le taux leucocytaire, la numération différentielle des globules blancs, et le poids des viscères. Le gain pondéral moyen (54,5 g / poisson), la longueur standard des nageoires et l'indice viscérotropique étaient significativement plus élevés chez les poissons ayant reçu un complément de 250 mg / kg de TP. Des changements significatifs ont été notés au niveau des paramètres sanguins des poissons traités par rapport aux poissons du groupe témoin. L'on peut conclure que les extraits des racines de *Tetracera potatoria* n'ont eu aucun effet toxique sur les poissons, et qu'ils peuvent servir de facteurs de croissance naturels dans la production aquacole et conduire à une productivité accrue.

Mots-clés : *Clarias gariepinus*, croissance, hématologie, *Tetracera potatoria*

Introduction

The international community faces great challenges in the coming decades including reining in global climate change, ensuring food security for the growing population, and promoting sustainable development. Changes in the agriculture sector are essential to meeting these challenges. Agriculture provides the main source of livelihood for the poor in developing countries, and improving agricultural productivity is critical to achieving food security as well as most of the targets specified under the Millennium Development Goals (1).

Aquaculture is the growing and cultivation of different species of fish including other aquatic animals for the purpose of feeding, decoration, ornamental and for advance research. This branch of agriculture has become very important being that they are good source of protein, vitamins, oil e.t.c. (2). Aquaculture fish production has been increased significantly over the past few decades which has led to intensive fish culture practices where stressors like overcrowding, transport, handling, size grading and poor water quality are common problem.

Clarias gariepinus is the most cultured fish in Nigeria and indeed Africa (3). Since the last three decades, clariid species has been considered to hold great interest for fish farming in Africa and Nigeria in particular. In order to improve health conditions in the rearing of aquatic organisms, several alternatives such as improved husbandry, nutrition, and water quality; optimal stocking density; and use of vaccines, probiotics and immunostimulant have been proposed (4). An immunostimulant is a substance that elevates non-specific defence mechanisms and specific immune response if the treatment is followed by vaccination or infection (5). In recent years, there has been growing interest in the field of herbal medicines research and search for promising potential area of investigating of immunomodulatory agents from natural products. Herbal drugs are believed to enhance the natural resistance of the body against infection and their immunomodulatory activities have been reported in numerous plants (6).

Tetracera potatoria Afzel, family Dillenniaceae is known as liane a eau in France and water tree in Sieerra-leone (7). It is found in wooded areas of Senegal, Southern part of Nigeria, Central and Eastern Africa (8) also known as Awo-Ekun in Nigeria. The leaves of the plant boiled in its own sap are used for the treatment of gastrointestinal sores (7). Adesanwo *et al.,* (9) reported the antiulcer activity of the methanolic extract of the root of *Tetracera potatoria*, and Oyebanji et al (10) reported the anti-inflammatory activity of this plant. This study examined the effects of *Tetracera potatoria* on the growth and non-specific immune response of catfish fingerlings as a means of increasing productivity.

Materials And Method

The experiment was conducted at the Faculty of Agriculture, Department of Animal Sciences Wet laboratory, Obafemi Awolowo University Ile-Ife Osun State located in the South West Nigeria.

Fingerlings of *Clarias gariepinus* were bought from hatcheries in Lagos State, and were transported in plastic containers to the site of the experiment. The *Clarias gariepinus* fingerlings were acclimated to experimental condition for 14 days at ambient room temperature prior to the feeding trial.

Preparation of Plant Extract and Fish Feed

Fresh roots of *Tetracera potatoria* were purchased at Bode Market, Ibadan in Southwestern Nigeria. The sample was authenticated at the herbarium unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife where voucher specimen was deposited.

The roots were oven-dried at 37oC and ground to powder forms after drying using hammer mill. Powdered and weighed samples of plant sample was extracted in 100% methanol by soaking for 72 hours. The resulting crude methanolic extracts (MeTP) was then concentrated under reduced pressure at 35°C in a rotary evaporator to obtain the solid

samples which was weighed and stored in the desiccator for pharmacological studies.

Surface-coating of feed

Known weight of extract was reconstituted with olive oil after which they were used to coat the feed and air-dried.

Experimental fish and feeding:

At the end of the acclimatization period, a total of 120 fingerlings were (mean initial weight 30 ±0.3 g) assigned into 6 groups (T1, T2, T3, T4, T5 and T6) and replicated twice.

- T1 Control,
- T2 Vitamin C @ 600mg/kg bw
- T3 *Tetracera potatoria* @ 250mg/kg bw,
- T4 *Tetracera potatoria* @ 500mg/kg bw,

The fish were kept in 45L rectangular plastic tanks. Each culture treatment was fed with durante (2mm) floating diet, a complete dry catfish food containing 45% of protein, 14% fat, 1.6% of calcium, 1% potassium, 7.6% Ash, 2.5% fiber, 60 ppm minerals and vitamins A 5000iu/kg, D 750mg/kg, E 150 mg/kg, and C 100mg/kg. Each experimental diet was randomly assigned to duplicate tanks. The fingerlings were fed 5% of their body weight with the respective diet twice daily, morning (8.00am – 10.00am) and evening (4.00pm – 6.00pm). During the trial, the water temperature was maintained at 29.0±2.0°C and Dissolved oxygen ranged from 3.10-6.13mgL-1. The experimental unit was under a natural light and dark cycle.The sampling of fish for weight and length measurement was done by reducing the volume of water with a rubber siphon before the fish were collected. The weighing was done per treatment and on a weekly basis. On weighing days, the fishes were not fed in the morning until the whole exercise was completed and fed in the late afternoon. The feeding trials lasted for eight weeks (56 days).

Statistical analysis:

Data obtained were subjected to one way Analysis of Variance (ANOVA) and the mean were separated using Duncan multiple range test. SAS package was used for this analysis.

Results and Discussion

There was a significant increase

Table 1: Effect of *Tetracera potatoria* on red blood cell parameters of catfish fingerling

Parameters	Control (T1)	Vit. C (T2)	Tp250 mg/kg (T3)	Tp500 mg/kg (T4)	SEM
PCV (%)	22.20^{b}	26.40^{a}	26.20^{a}	24.90^{a}	1.21
HB (g/dl)	7.29^{ab}	8.69^{a}	8.67^{a}	8.22^{a}	0.41
RBC(x106/l)	2.25^{c}	2.47^{bc}	3.19^{a}	2.98^{ab}	0.15
Thrombocyte (1000/mm2)	7.40^{b}	8.10^{b}	13.64^{a}	7.60^{b}	1.10
MCV(fl)	92.50^{a}	85.70^{ab}	85.80^{ab}	86.90^{a}	2.09
MCH (pg)	32.40^{a}	34.80^{a}	28.60^{b}	28.50^{b}	1.30
MCHC (pg)	33.0	33.0	33.0	33.0	0

Key: (PCV) Packed cell volume, HB-hemoglobin, RBC- red blood cell, WBC- white blood cell, MCV- Mean corpuscular volume, MCH-Mean corpuscular haemoglobin, MCHC- Mean corpuscular haemoglobin concentration,

Table 2: Effect of *Tetracera potatoria* on differential white blood cell count of catfish fingerling

Parameters	Control (T1)	Vit. C (T2)	Tp250 mg/kg (T3)	Tp500 mg/kg (T4)	SEM
WBC(x103/µL)	14.38^{a}	13.34^{a}	14.00^{a}	10.98^{b}	0.61
LYMP (%)	68.00^{b}	65.50^{b}	74.60^{a}	61.20^{c}	2.32
NEUT (%)	30.90^{b}	34.10^{ab}	23.90^{c}	37.90^{a}	2.42
MONO (%)	1.20^{b}	1.40^{a}	1.40^{a}	1.30^{ab}	0.05

Key: LYMP- Lymphocyte, Neut- Neutrophil, MONO- monocyte. Means with different superscript are significantly different at p<0.05

Table 3: Effect of *Tetracera potatoria* on growth parameters of catfish fingerling

Parameters	Control (T1)	Vit. C (T2)	Tp250 (T3)	Tp500 (T4)	SEM
Initial weight (g)	30.0	30.0	30.0	30.0	0
Final weight (g)	75.0^{b}	65.5^{c}	84.5^{a}	71.5^{b}	4.61
Final length (cm)	20.3^{a}	19.5^{a}	21.8^{a}	20.5^{a}	0.70
Condition factor (CF)	3.7^{b}	3.4^{b}	3.9^{a}	3.5^{b}	0.12
Mean weight gained (g)	45.0^{b}	35.5^{c}	54.5^{a}	41.5^{b}	5.02
% Weight Gained	150^{b}	118^{c}	182^{a}	138^{b}	13.54
% Survival	80^{b}	100^{a}	100^{a}	100^{a}	3.33

Means with different superscript are significantly different at p<0.05

Table 4: Effect of *Tetracera potatoria* on growth indices of catfish fingerling

Parameters	Control (T1)	Vit. C (T2)	Tp250 mgkg- (T3)	Tp500 mgkg (T4)	SEM
Visceral	6.9^{b}	4.0^{c}	9.7^{a}	7.8^{ab}	1.07
Liver	0.9^{ab}	0.5^{bc}	1.28^{a}	1.05^{ab}	0.15
VSI	9.4^{ab}	6.7^{c}	10.4^{a}	8.59^{ab}	0.57
HSI	1.1^{a}	0.8^{b}	1.21^{a}	1.12^{a}	0.09

VSI =Viscerosomatic Index, HSI= Hepatosmatic Index.
Means with different superscript are significantly different at p<0.05.

(p<0.05) in the PCV, HB and RBC of fish in the treatment groups except PG500 group when compared with the control. TP250 had the highest values with PCV of 26.20%, hemoglobin count of 8.67g/dl and RBC of 2.54x106/l respectively when compared with the control group (Table1).

There was significant decrease (p<0.05) observed in the WBC values of TP500 (10.98x103/µl) animals (Table 2). The growth response of *Clarias gariepinus* fingerlings fed with experimental diets for 56 days weeks are presented in Table III. The best weight gain (54.5g) of African catfish fed with 250mg/kg of *Tetracera potatoria* was significantly (p<0.05) higher than that of other groups in the experiment. The growth indices were significantly higher for fish treated with TP250 as compare with the control group (Table IV).

No mortalities were noticed in the extract incorporated diet fed groups throughout the experimental period.

Discussion

Analysis of blood has been developed and well utilized in assessing the health of man and lifestock (11). Svesbodora (12) reported that ichthohaemology would be useful in the assessment of suitability of feeds and feed

mixture evaluation of substances as well as a diagnosis of disease. In fish blood, oxygen is carried in combination with haemoglobin and this is very important for the survival of the fish. The significant increase in the haematological parameters in the treatment groups may be due to the presence of flavonoids in *T. Potatoria* (13) which may maintain the heam iron in its ferrous state and thus enhance erythropoiesis (14). This is similar to the observation of Sahu et al (15), where the dietary intake of mango kernel by Labeo rohita juveniles had significantly ($P<0.05$) increased RBC in all the treatment groups as compared with control group. Many other studies with different plants like Coriandrum sativum (16) and Plumbago rosea (17) Cynodon dactylon in Catla catla (18,19) ,in Cyprinus carpio treated with Epilobium hirsutum mixed diet (20) and in Oreochromis mossambicus treated with Andrographis paniculata. Prasad and Mukthiraj (21) confirmed the ability of medicinal plants to enhance haematological parameters of fish. There was significant difference in the WBC of the animals fed with TP500mg/kg. Inspite of the differences the WBC for all the group fell within the normal range reported for C. fingerling which according Adedeji and Adegbile (22) was 10.6 – 36.2x103/µl.

Growth promoters are commonly added to animals feed for growth enhancement and efficient feed utilization. They are chemical products, antibiotics, enzymes and/or natural extractives. Since the use of chemical products antibiotics might have some unfavorable side effects, therefore researchers tended to use natural additives which meet the requirements of good growth promoting agents (23). There was a general increase in weight gain of the fish in the course of the experiment with the highest growth performance observed in fish fed 250mg/kg of *Tetracera potatoria* with mean weight gain of 54.5g and mean length of 21.76cm . The condition factor, hepatosomatic index and viscerosomatic index were used to assess the nutritional and physiological status of experimental fish. Fish fed diet with *Tetracera potatoria* (250mg/kg) had the highest hepatosomatic and viscerosomatic indices than the rest of treatments, this may be due to enhanced development of liver and spleen which are the main blood forming organs in fish (in addition to the fore-kidney) this is similar to the result recorded by Yasser *et al.,* (24) where wormseed plants and chamomile fed to catfish enhanced the hepatosomatic and splenosomatic indices. Hepatosomatic Index (HSI) is defined as the ratio of liver weight to body weight. It provides an indication on status of energy reserve in an animal. In a poor environment, fish usually have a smaller liver (with less energy reserved in the liver).

In fisheries science, the condition factor is used in order to compare the "condition" and "fatness" or well being of fish. It has been hypothesized that heavier fish of a particular length are in a better physiological condition (25).

Condition factor is also a useful index for the monitoring of feeding intensity, age, and growth rates in fish (26). It is strongly influenced by both biotic and abiotic environmental conditions and can be used as an index to assess the status of the aquatic ecosystem in which fish live (27). In the present study increased condition factor was noticed in TP250 treatment group when compared to initial condition factor of fish. Condition factor of African catfish fed the diet containing extract might have provided optimal nutrients for fish.

The fish in the treatment groups had 100% survival rate as against 80% in control group meaning that the exposure of C. gariepinus fingerlings to the plant extract at graded doses was not toxic and could have acted to reduce stress on the fish. This is in agreement with the work of Kurva and Dash (28) that natural plant products promote various activities such as antistress, growth promotion, immunostimulation due to the presence of active principles such as flavonoids, essential oil, steroids etc.

In conclusion, *Tetracera potatoria* methanolic root extract inclusion at 250mg/kg into feed of *Clarias gariepinus* fingerling has a growth enhancing effect, and is therefore recommended as a means of increasing productivity.

References

Rosegrant ,W. 2009. Building climate resilience in the agriculture sector. Manila, Philippines and Washington, D.C.: Asian Development Bank and International Food Policy Research Institute.

Solomon, J.R and Ezigbo, M. N 2010. New York Science Journal : 3(10) 42

Soosean, C. Marimuthu, K., Sudhakaran, S and Xavier, R. (2010). Eur Rev Med Pharmacol Sci.14(7):605

Prasad, G and Mukthiraj, S (2011). World J. Fish and Marine Sci. 3 (6): 473

Siwicki, A.K. and M. Korwin-Kossakowski (1988). Journal of Applied Ichthyology 4: 178.

Upadhaya, S.N. (1997). Therapeutic Potential of Immunomodulatory Agents from Plant Products. 1st Edn., Narosa Publishing House, New Delhi, pp: 149-150.

Burkill, H.M. (1985): The useful plants of West Tropical Africa. 2nd Edition. Royal Botanic Gardens, Kew. 1:650-652.

Dalziel, J.M (1937): The useful parts of West Tropical Africa. London: Crown Agent Publication

Adesanwo, J.K; Ekundayo, O., Oluwole, F.S., Olajide, O.A., Van Den Berge, A.J. and Findlay J.A (2003). Nigeria Journal on Physiology. Sci. 22:21

Oyebanji B.O, Saba A.B and Oridupa O.A . (2013). Afr. J. Biomed. Res. Vol.16; 199

Adeparusi, E.O,Ajayi A. D. (2003). Hematological characteristics ofNile Tilapia (Oreochromis niloticus)fed differentlyprocessed lima Bean (Phaseolus lunatusl.) Diets. Fisheries Wildlife Department, Federal University of Technology.

Svesbodora, Z., Fravda, D. and Palakova,J.(1991). Unified Methods of Haematological Examination of Fish. Research Institute of Fish Culture & Hydrolobiology, Vodnany, Czechoslovekia. 331

Oyebanji, B.O and Saba, A.B. (2011). Journal of Medicinal plant research Vol. 5(30), 6622

Shatoor, A.S. (2011). American Journal of Pharmacology and Toxicology,; 6: 37

Sahu, S, Dasb, K, Mishrab, K, Pradhan, J, Sarangi, N. (2007). J Appl Ichthyol ; 23: 80

Innocent, B.X., Fatima, M.S.A, Dhanalakshmi (2011a). Journal of Applied Pharmaceutical Science 1: 132.

Innocent, B.X, Fatima, M.S.A, Siva Rajani, S. (2011b). International Journal of Applied Pharmaceutical Technology; 2: 447-454.

Kaleeswaran, B, Ilavenil, S, Ravikumar, S. (2010a). Journal of King Saud University (Science) ; 24:139.

Kaleeswaran, B., Ilavenil, S., andRavikumar, S. (2012b). Hitek J. Bio. Sci. and Bioengg ; 1:1.

Pakravan, S., Hajimoradloo, A. and Ghorbani, R.(2012). Aquaculture research ; 43: 861.

Prasad, G. and Mukthiraj, S.(2011). World J. Fish and Marine Sci. 3 (6): 473

Adedeji O.B. and Adegbile A.F (2011). Journal of Applied Sciences Research, 7(7): 1042

Flück,H., R. Jaspersen-Schib and J.M. Rowson, (1976) . Medicinal plants and their uses. W. Foulsham and Co. Ltd., Berks, U.K., pp: 188

Yasser M.A, Osama A. S and Saleh F. S. (2012). Journal of Fisheries International. 5 : (1) 1

Kumuolu-Johnson, C.A and Ndimele, P.E (2010). Asian Journal of Agricultural Sciences 2(4):174

Oni,S.K., J.Y. Olayemi and J.D. Adegboye, (1983). J.Fish Biol ., 22:105

Anene,A. (2005). Turk. J.Fish.Aquat. Sci., 5:43

Kurva, R.R and Dash, G. (2013). .American Journal of Drug Discovery and Development ISSN 2150-427x

GENITALIA MORPHOMETRY AND TESTICULAR CHARACTERISTICS OF MALE WHITE JAPANESE QUAILS AT THREE DIFFERENT AGE GROUPS

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Abstract

An experiment was designed to evaluate genitalia morphometry of the male white Japanese quails at three different age groups. Fifty-four male Japanese quails were allotted to 3 treatment groups (Pubertal, Mature and Adult) in a completely randomized design. Pubertal (7-10 weeks), mature (15-20 weeks) and the adults (≥ 24 weeks). The initial weight of the quails was taken. All the animals were sacrificed and organs were carefully excised. The total length of reproductive tract and sections of the tract of male quails was taken. Total weight of reproductive tracts, right and left testicular weight, right and left epididymis, right and left testicular diameter and circumference were determined. The weight of the male genitalia tract of white Japanese quails was similar across different age groups. The length of the genitalia tract was significantly higher in the pubertal group than the adult and mature groups. Testicular circumference and diameter of white Japanese quails at puberty was significantly ($P<0.05$) higher than at adulthood. It was concluded that the male pubertal quail have well developed reproductive tracts and thus could have potential for high reproductive ability similar to later physiological ages. Farmers can do more breeding activity when the birds are at the pubertal age.

Keywords: Reproductive tract, White quails, Testicular characteristics, Quail epididymis

MORPHOMÉTRIE DES ORGANES GÉNITAUX ET CARACTÉRISTIQUES TESTICULAIRES DES CAILLES JAPONAISES BLANCHES MÂLES APPARTENANT À TROIS GROUPES D'ÂGE DIFFÉRENTS

Resume

Une expérience a été conçue pour évaluer la morphométrie des organes génitaux des cailles japonaises blanches mâles appartenant à trois groupes d'âge différents. Cinquante-quatre cailles japonaises mâles ont été réparties à 3 groupes de traitement : (pubères, matures et adultes) dans un dispositif complètement aléatoire : Pubères (7-10 semaines) ; Matures (15-20 semaines) ; et Adultes (≥ 24 semaines). Le poids initial des cailles a été enregistré. Tous les animaux ont été sacrifiés, et leurs organes ont été soigneusement excisés. La longueur totale de l'appareil reproducteur et des sections du tractus reproductif des cailles mâles ont été mesurées. Le poids total des appareils reproducteurs, le poids des testicules gauche et droit, les épididymes des testicules droit et gauche, et le diamètre des testicules gauche et droit ainsi que leur circonférence ont été déterminés. Le poids de l'appareil génital mâle des cailles blanches japonaises était similaire pour les différents groupes d'âge. Le tractus génital était significativement plus long dans le groupe pubère par rapport aux groupes adultes et matures. La circonférence et le diamètre des testicules des cailles japonaises blanches pubères étaient significativement ($p <0,05$) plus élevés par rapport aux cailles adultes. Il a été conclu que les cailles pubères mâles avaient un appareil reproducteur bien développé et pourraient donc avoir un potentiel de reproduction élevé similaire à celui des âges physiologiques ultérieurs. Les pisciculteurs peuvent mener des activités supplémentaires d'élevage lorsque les oiseaux ont atteint l'âge de la puberté.

Mots-clés : appareil reproducteur, cailles blanches, caractéristiques testiculaires, épididyme de la caille

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Introduction

Knowledge of the genitalia morphometry of male and female species is very important as this can be applied in the production of such species for increased production and optimization of yield. Morphometric analysis on the testis of any species or breed is necessary in assessing and estimating quantitative changes in testicular components and spermatogenic function arising from factors such as age, season, temperature and diseases (Egbunike *et al.,* 1976). Nutrition also exerts some influence on testicular morphometric parameters and on gonadal sperm reserves in Corriedale rams (Bielli *et al.,* 1997).

Gage and Freckleton (2003) described the mammalian testes as infallible predictors of spermatozoa production. It was further asserted that the knowledge of the basic morphometric characteristics of the reproductive organs is mandatory for assessment and prediction not only of sperm production but also of the storage potential and fertilizing ability of the breeder male. In mammalian species significant correlations have been reported between paired testes weight and body weight, sperm production and reserve potentials in boars (Gbore and Egbunike, 2008). Changes in testicular morphometry and sperm reserves due to seasons have been observed in domestic cats (Franca and Godinho, 2003) and camel (Al-Qarawi *et al.,* 2001).

A few studies have reported that there is a correlation between testicular growth and the body weight of fowls (Kumaran and Turner, 1949). However, Marvan (1969), Tingari et al. (1980) and Aire (1982) have also tried to establish a correlation between the sexual maturity of fowls, testicular development, testicular weight and the age of birds. Artoni (1993) described the testicular microanatomy and morphometry in quails (Coturnix coturnix japonica) and established the annual testicular cycle in this bird. Hess et al. (1976) described the ductus succession from the seminiferous tubule to the ductus deferens papilla, as well as the microanatomy of the epididymal region and the ductus deferens in the turkey (Meleagris gallopravo). On the other hand, Reviers (1971), by studying the testis development of hybrid Rhode x Wyandotte, reported the ponderal growth of the testis with the use of organ weight and histological analyses through measurement of the diameter of the seminiferous tubules.

The entire reproductive systems of the birds are necessary for breeding, but the testes, epididymis and ductus deferens are the most important functional regions. The male reproductive system in male birds consists of the testes, epididymis, ductus deferens, ejaculatory region and mating organ. Lately, researchers have taken into consideration studies on birds since they represent an excellent nutritional source. There are several classical descriptions of the male reproductive tract, always aiming at establishing a correlation with shape, testicular size, age and sexual maturity (Bull *et al.,* 2007). Experimental studies in connection to the female genitalia morphometry of avians comprising the Japanese quail, domestic fowl, turkey and duck have been conducted in relation to weight (Kashmiri and Vatsalya, 2011), the effect of feeding different dietary nutrient levels (Akinola *et al.,* 2012), genotype and the effect of cage systems and feeding time (Huseyin *et al.,* 2006).

The objective of this study was to assess the morphometry of male quails at three different physiological ages and to evaluate the effect of physiological age on the reproductive tracts of white male Japanese quails.

Materials and Methods

Experimental site

The experiment was carried out at the quail section of the poultry unit of the Teaching and Research Farm, University of Ibadan and the Animal physiology laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental animals and their management

Fifty-four white male Japanese quails were used for this experiment. They were purchased from the quail section of the poultry unit at the Teaching and Research Farm University of Ibadan and raised in floor

pens. Throughout the experiment, adequate provision of feed and water was made available ad libitum. The male quails within the three physiological age groups were on similar diet from day old. Animals were selected from the groups sacrificed. Their reproductive organs were taken out and tagged. The lengths and weights of the organs were taken in the laboratory.

Experimental layout

The white male Japanese quails were allotted to 3 treatments (age groups) of 18 birds per treatment. Each treatment had 3 replicates with 6 birds per replicate.

Treatment 1: Pubertal male white quails (7-10 weeks old)

Treatment 2: Mature male white quails (15-20 weeks old)

Treatment 3: Adult male white quails (≥ 24 weeks old)

Genitalia morphometry assessment of male quails

Quails at the different age groups were selected, weighed and sacrificed. Their reproductive tract and organs (testes and epididymis) were carefully excised and weighed using analytical weighing balance. The values were recorded in grams.

The length of the whole reproductive tract, length of the testis and epididymis were measured using thread. The piece of thread used for the measurement was then placed on a ruler and the reading was then taken and recorded in cm.

Testicular volume: This was determined by pouring a known quantity of distilled water into a measuring cylinder and dropping the testis into it. Using the Archimedes principles of water displacement, the quantity of water displaced in the cylinder was taken as the volume of the testis (Akinyemi *et al.,* 2014).

Testicular Density: This was calculated from the weight and volume of the testis.

$$\text{Testicular density} = \frac{\text{Mass/Weight of the testis}}{\text{Testicular volume}}$$

Testicular circumference: This was determined by using a piece of thread to measure the circumference of the testis by folding the thread around it and then placing it on a ruler to read.

Testicular diameter: The piece of thread was used to measure one side of the testis breadth-wise and was then placed on a ruler after which the reading was taken.

Statistical analysis

Data collected were analysed using correlation analysis and One-Way Analysis of Variance (ANOVA) procedure of SAS (2003) and means were separated using Duncan Multiple Range Test procedure of the same software.

Results

Length of genital tract of white male quails at different age groups

The length of the genitalia tracts of white male Japanese quails at different age groups is shown Table 1. It was observed that there was no significant difference in the length of the right tract among the treatments. The longest right tract was recorded in the pubertal group (6.41±1.49 cm) while the shortest right tract was recorded in the adult group (5.82±0.75 cm). There was however no significant difference in the left testicular length of the pubertal group and that of the mature group (2.17±0.49 cm). The pubertal group had significantly ($P<0.05$) higher epididymal length (10.06±2.76 cm) than the adult group (8.69±1.18 cm). However, there was no significant difference between the average epididymal length of the mature group (8.94±1.52 cm) and that of the adult group (8.69±1.18 cm).

Weight of the genital tract of white male quails at different age groups

The weight of genitalia tracts of white male quails at different age groups is shown in Table 2. It was observed that there was no

significant difference in the weight of the right tract among the physiological age groups in quails. It was also observed that there was no significant difference in the weight of the left tract, right testis and paired epididymal weight among the different age groups.

Testicular volume, circumference and diameter of white male quails at different age groups

The testicular volume, testicular circumference and testicular diameter of the white male Japanese quails is shown in Table 3. It was observed that there was no significant difference in the right and left testicular volume among the treatments. The pubertal male quails had a significantly (P<0.05) higher right testicular circumference (4.24±0.71 cm) than the adult male quails (3.34±0.97 cm). There was however no significant difference between the right testis circumference of the adult group and that of the mature group (3.90±0.89 cm). There was significant (P<0.05) difference in the left testis diameter as influenced by age group. The pubertal group had significantly (P<0.05) higher left testis diameter than the adult group (1.7±0.49 cm). There was however no significant difference between the left testis diameter of the adult group and that of the mature group.

The correlation coefficient between live weight and reproductive organ weight, circumference and diameter of white male Japanese quails.

The correlation analysis of some selected reproductive organs and live weight in white male quails is as shown in Table 4. It was observed that paired testis volume had a significant and strong positive correlation with right testis volume (r= 0.92; P<0.01). Paired testes circumference also was observed to have a significant, strong and positive correlation with right and left testicular circumference (r= 0.91; P< 0.01). The live weight of the quails had significant, and positive correlation with the left testicular circumference (r= 0.56; P< 0.01). There was also a non-significant, but positive correlation between the paired testes volume and the paired testes circumference (r= 0.30; P>0.05).

Discussion

Several reports have been documented establishing varying weights of reproductive organs in relation to age at sexual maturity (Miclea *et al.,* 2002; Li *et al.,* 2006). Physiological age group significantly influenced the testicular morphometry in the male white quails. The significantly higher reproductive tract in the pubertal group could be attributed to the higher level of circulating testosterone, luteinizing hormone and follicle stimulating hormone in the pubertal birds. It has been documented that sexual capacity reduces in animals as they grow older (Gonzalez-Moran and Soria-Castro 2010).

In vitro experiments have shown the β-endorphin and met-enkephalin inhibited GnRH-1 release from quail hypothalamic slices (Ottinger, 1998) in all groups of quails but this was less pronounced in younger and senescent animals compared to adults. However, in the

Table 1: Length of genitalia tracts of white male quails at different age groups

Parameters	Pubertal 7-10 weeks	Mature 15-20 weeks	Adult ≥24 weeks
Right Tract (cm)	6.41±1.49	6.12±0.77	5.82±0.75
Left Tract (cm)	6.37±1.41	6.01±0.81	5.63±0.87
Average Tracts (cm)	12.78±2.50^{a}	12.13±1.42^{ab}	11.45±1.41^{b}
Right Testis (cm)	2.26±0.61	2.33±0.66	1.99±0.43
Left Testis (cm)	2.33±0.49^{a}	2.17±0.49^{ab}	1.92±0.33^{b}
Average Testis (cm)	4.59±1.04^{a}	4.49±0.98^{a}	3.81±0.78^{b}
Right Epididymis (cm)	4.74±1.55^{a}	4.71±0.85^{b}	4.46±0.68^{a}
Left Epididymis (cm)	5.32±1.47^{a}	4.24±0.82^{b}	4.23±0.57^{b}
Average Epididymis (cm)	10.06±2.76^{a}	8.94±1.52^{ab}	8.69±1.18^{b}

^{a,b}: Means in the same row with different superscripts are significantly (p<0.05) different

Table 2: Live weight, weight of genital tracts and organs of white male quails at different age groups

Parameters	Pubertal 7-10 weeks	Mature 15-20 weeks	Adult ≥24 weeks
Right Tract (g)	2.33±0.58	2.23±0.53	2.49±0.59
Left Tract (g)	2.57±0.72	2.22±0.53	2.46±0.52
Paired Tract (g)	4.90±1.24	4.46±1.04	4.95±1.09
Right Testis (g)	2.06±0.59	2.06±0.53	2.41±0.81
Left Testis (g)	2.23±0.59	2.04±0.55	2.41±0.69
Paired Testes (g)	4.28±1.14	4.11±1.04	4.81±1.49
Right Epididymis (g)	0.12±0.04	0.12±0.04	0.13±0.05
Left Epididymis (g)	0.14±0.05	0.12±0.04	0.12±0.04
Paired Epididymis (g)	0.26±0.08	0.23±0.06	0.24±0.08
Live weight (g)	133.57±3.64[a]	127.57±10.21[b]	130.7±6.32[ab]

[a,b]: Means in the same row with different superscripts are significantly ($p<0.05$) different

Table 3: Testicular Volume, circumference and diameter of white male quails at different age groups

Parameters	Pubertal 7-10 weeks	Mature 15-20 weeks	Adult ≥24 weeks
Right Testis Volume (cm^3)	1.94±0.64	1.67±0.49	1.50±0.86
Left Testis Volume (cm^3)	2.11±0.90	1.72±0.57	1.64±0.76
Paired Testes Volume (cm3)	4.06±1.43	3.39±1.04	3.14±1.49
Right Testis Circumference (cm)	4.24±0.71[a]	3.90±0.89[ab]	3.34±0.97[b]
Left Testis Circumference (cm)	4.44±0.85[a]	3.90±0.92[ab]	3.40±0.97[b]
Paired Testes Circumference (cm)	8.69±1.46[a]	7.84±1.79[ab]	6.74±1.92[b]
Right Testis Diameter (cm)	2.12±0.35[a]	1.95±0.45[ab]	1.67±0.49[b]
Left Testis Diameter (cm)	2.25±0.41[a]	1.97±0.46[ab]	1.7±0.49[b]
Average Testes Diameter (cm)	4.37±0.72[a]	3.92±0.89[ab]	3.37±0.96[b]

[a,b]: Means in the same row with different superscripts are significantly ($p<0.05$) different

current study, it was observed that physiological age group significantly influenced testicular and epididymal parameters. Miclea et al. (2002) and Li et al. (2006) have reported that a close relationship exists between the weight of testis and age at sexual maturity, as controlled by factors such as genetics, body weight (Broody *et al.,* 1980), chronological age, environmental and chemical composition of the animals (Zelanka *et al.,* 1984) and nutrition (Hashiguishi *et al.,* 1998).

Physiological age group significantly influenced the live weight of the birds. Pubertal group's higher live weight could be attributed to higher feed efficiency/conversion occurring in the birds. Also a higher metabolic rate could be attributable to the observed difference in the 3 age groups of the animals. Younger animals have higher tendencies to eat more as they are still capable of growing while older animals are not capable of growing at the same rate.

Physiological age group did not influence the weight and length of the genital tract of the quails. The weight and length of the right tract were not significantly influenced by the physiological age groups. This supports the findings of Ipek et al. (2003) who observed that breeder pairs distributed under 3 lighting conditions with respect to age did not affect organ morphometry in the experimental birds.

Table 4: Correlation coefficient of live weight and the volume, circumference, diameter, and weight of reproductive organs of male Japanese quails

Parameters	Right testis vol. (cm^3)	Left testis vol (cm^3)	Paired testes vol (cm^3)	Right testis C (cm)	Left testis C (cm)	Paired testis C (cm)	Right testis D (cm)	Left testis D (cm)	Paired testis D (cm)	Live Weight (g)	Total tract L (cm)	Total tract W (g)	Right testis W (g)	Left testis W(g)	Paired testis W (g)
RTV(cm3	1	.733**	.921**	.518**	.475**	.574**	.409**	.464**	.474**	.259	.349**	.248	.124	.121	.162
LTV (cm3)		1	.941**	.479**	.494**	.556**	.420**	.465**	.511**	.259	.207	.213	.348**	.330*	.346*
PTV (cm3)			1	.534**	.521**	.606**	.446**	.499**	.530**	.278*	.251	.173	.213	.182	.212
RTC (cm)				1	.844**	.917**	.761**	.788**	.929**	.363**	.342*	.423**	.335*	.271*	.289*
LTC (cm)					1	.915**	.710**	.819**	.909**	.567**	.399**	.338*	.201	.252	.189
PTC (cm)						1	.765**	.803**	.936**	.443**	.396**	.427**	.345*	.271*	.307*
RTD (cm)							1	.797**	.728**	.469**	.148	.216	.131	.077	.125
LTD (cm)										1	.762**	.443**	.179	.129	.100
PTD (cm)									1	.466**	.162	.180	.114	.065	.097
LW(g)										1	.764**	.826**	.794**	.647**	.706**
TTL (cm)											1	.859**	.561**	.620**	.569**
TTW (g)												1	.694**	.713**	.736**
RTW (g)													1	.828**	.873**
LTW (g)														1	.868**
PTW (g)															1
PTW (g)															1

** - Significant (P<0.05), ** -Very significant (P <0.01).Vol-volume, C-Circumference, D-Diameter, L- Length,W-Weight, RTV-Right testis volume, LTV-Left testis volume, PTV-paired testis volume, RTC-Right testis circumference, LTC-Left testis circumference, PTC-Paired testis circumference, RTD-Right testis diameter, LTD-Left testis diameter, PTD-Paired testis diameter, LW-Live weight, TTL-Total tract length, TTW-Total tract weight, RTW-Right testis weight, LTW-Left testis weight, PTW-Paired testis weight.*

Testicular volume was not significantly influenced by the three physiological age groups. This may probably be as a result of the early sexual maturity that quails attain very early in life. However, paired testes diameter and paired testes circumference, were all significantly influenced by the physiological ages. Several reports have documented a decline in sexual characteristics in poultry as they grow older suggesting that younger birds are more sexually active. Several studies have demonstrated that older animals that attain sexual maturity experience a quick decline in fertility.

Significant, positive but weak correlation between paired testicular circumference and weight of total tract is suggestive of the fact that the testes develop independent of what happens to the reproductive accessory organs. Several studies have established that age and body weight of an animal determines the efficiency of development of the reproductive organs (Osinowo *et al.,* 1981; Ewuola and Egbunike, 2010).

Conclusion

In this study, physiological age group did not influence the weight and length of the genital tract of the quails. However, testicular dimensions were highest at puberty compared to the mature and adult age groups. Pubertal male quails had better reproductive organ development than others, and thus could have potential for high reproductive ability compared to the other age groups. Farmers can do more breeding activity when the birds are at the pubertal stage.

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported in this study.

References

Aire TA. 1982. The rete testis of birds. J. Anat., 135(1): 97-100.

Akinola LAF, Sese BT, Oruwari BM. 2012. The effect of feeding different dietary nutrient levels on sexual maturity of Japanese quail (Cortunix Cortunix Japonica) J. Anim. Prod. Adv., 2(11): 500-506.

Akinyemi MO, AJ Aina, EO Ewuola, OH Osaiyuwu and EO Ajao. 2014. Relationships between body morphometrics and testicular biometrics of West Africa dwarf bucks in Southwestern Nigeria. Inter J Agri Biosci, 3(6): 283-287.

Al–Qarawi AA, HA Abdel-Rahman, MS El-Belely, SA El-Moug, 2001. Intratesticular morphometric, cellular and endocrine changes around the pubertal period in dromedary camels Vet. J., 162: 241-249.

Artoni SMB. 1993) Consideracoes sobre a morfologia e a histofisologia do testiculo da codorna (Cortunix cotunix japonica). Botucatu-sao Paulo. Tese (Doutorado em Anatomia)- Instituto de Biociencias, Universidade Estadual Paulista.

Bielli AT, Gastel R, Perez A, Lopez and A Castillejo. 1997. Influence of nutrition on seasonal variations in testicular morphology and function in Corriedale rams. J. Reprod. Dev., 43: 171-180.

Broody T, Eltan Y, Soller M. Nir I. and Nitsan Z. 1980. Compensatory growth and sexual maturity in broiler females reared under severe food restriction from day of hatching. British Poultry Sci, 21, 437-446.

Bull LM, Fernandas MR, Martins B, Cesario DM, Pdovani R. Mendes AA. 2007. Anatomical study on domestic fowl (Gallus domesticus) reproductive system. Int. J. Morphol, 25(4): 709-716.

Egbunike GN, Holtz W, Smidt D. 1976. Reproductive capacity of German Landrace boars. II: Sperm production rates as determined by quantitative testicular histology and fron gonadal sperm reserves. Zuchthygiene, 11:35-7.

Ewuola EO, Egbunike GN. 2010. Gonadal and extra-gonadal sperm reserves and sperm production of pubertal rabbits fed dietary fumonisin B1. Journal of Animal Reproduction Science, 119: 282-286.

Franca LR, Godinho CL. 2003. Testis morphometry, seminiferous epithelium cycle length and daily sperm production in domestic cats (Felis catus). Biol. Reprod., 68: 1554-1561.

Gage MJG. Freckleton RP 2003. Relative testis

size and sperm morphometry across mammals: No evidence for an association between sperm competition and sperm length. Proc. Biol. Sci., 22: 625-632.

Gbore FA, Egbunike GN. 2008. Testicular and epididymal sperm reserves and sperm production of pubertal boars fed dietary fumonisin B1. Vol. 105, Issues 3-4, Pg 392-397.

Gonzalez-Moran MG, Soria-Castro E. (2010). Histological and stereological studies on Leydig cells in the testes of Gallus domesticus from pre-hatching to sexual maturity. Anim. Reprod. Sci., 120: 129-135.

Hashiguishi, M., Mahipala M.B.P., Kamisoyama H. (1998). Effect of low protein diets on sexual maturity and cody characteristics in female Japanese quail. Proceedings of 6th Asian Poultry Congress. Pp. 408-409.

Hess RA, Thurston RJ, Biellier HV. 1976. Morphology of the epididymal region and ductus deferens of the turkey (Meleagris gallopravo). J. Anat. 124, 765-778.

Huseyin Y, Yilmaz B, Arican I, Petck M, Bahadir A. 2006. Effect of cage systems and feeding time on the morphological structure of female genital organs in Pharaoh quails (Cortunix cortunix pharaoh). Veterinary Archive 76, 381-389.

Ipek, A, Sahan U, Yilmaz B. 2003. The effect of ovo ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. Arch. Geflu¨gelk. Verlag Eugen Ulmer GmbH and Co., Stuttgart 68(3): 132-135.

Kashmiri LA, Vatsalya V. 2011. Association between body weight growth and selected physiological parameters in Japanese male quails. Int. J. Poult. Sci. 10(9): 680-684.

Kumaran LDS, Turner CW. 1949. The normal development of testis in the white Plymouth rock. Poult. Sci., 28: 511-20.

Li C, Takahashi S, Taneda S, Finata C, Watanabe G, Suzuki AK, Taya K. 2006. Impairement of testicular function in male Japanese quail (Cortunix japonica) after a single administration of 3-methyl-4-nitrophenol in diesel exhaust particles. Journal of Endocrinology. 189, 555-564.

Miclea V, Ladosi D, Ladosi I, Zaham M. 2002. The influence of inducing early reproduction activity in young hen and roosters of egg laying type breed. Journal of Central European Agriculture 3(4): 353-362.

Osinowo OA, Buvanendram V, Koning NL. 1981. Growth and testicular development in Bunaji bulls. J. Anim. Prod. Res. 1, 55-67.

Ottinger MA. 1998. Male reproduction: testosterone, gonadotropins, and aging. In: Mobbs CV, Hof PR (eds) Functional Endocrinology of aging, Vol 29, pp 105-126.

Reviers M. 1971. Le développement testiculaire chez le coq. I –Croissance ponderale des testicules et développement des tubes séminifères. Ann. biol. anim. Bioch. Biophys., 11(4):519-30.

SAS. 2003. Statistical Analysis System . SAS stat. Version 9. SAS Institute Inc. Gary NC U.S.A.

Tingari KA, Kunzel MD, Moniem 1980. The fine structure of the boundary tissue of seminiferous tubule of the camel (Camelus dromedarius). Acta Anat. 107: 169-176.

Zelanka DJ, Cherry JA, Nair I, Siegal PB. 1984. Body weight and composition of Japanese quail (Cortunix Japonica) at sexual maturity and growth. 48: 16-28.

EFFECT OF RED AND YELLOW GINGER ON GROWTH PERFORMANCE AND TOTAL ANTIOXIDANT CAPACITY OF BROILER CHICKEN

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Abstract

The objective of this study was to compare the effect of the red and yellow ginger on performance, nutrient digestibility and total antioxidant capacity of broiler chickens. In a 2×2×2+1 factorial design, a total of two hundred and sixteen, three weeks old broiler chicken with initial weight of 430 g (± 0.1g SE) were assigned to nine dietary treatments (TRT) and each treatment consisted of 3 replicates of 8 birds per replicate each. The factors were two ginger types, processed by two methods of sun-drying (SD) and oven-drying (OD) at two inclusion levels of 1.6% and 1.8% and a non-supplemented control diet. Treatment I was the control and contained no ginger, while TRT II, III, IV and V contained red ginger at 1.6% (OD), 1.8% (OD), 1.6% (SD) and 1.8 (SD) respectively. Treatments VI to IX contained yellow ginger at 1.6% (OD), 1.8% (OD), 1.6% (SD) and 1.8 (SD) respectively. The performance of broilers was significantly affected by ginger type with birds on red ginger having higher ($p < 0.05$) final body weight than birds on yellow ginger (2019 vs. 1915 g/bird). The apparent digestibility of the diets for most proximate constituents was significantly higher for red than yellow ginger. The total antioxidant capacity determined cupric reducing antioxidant capacity was significantly higher for red than yellow ginger, while that determined by total radical-trapping antioxidant parameter was higher ($p < 0.05$) for yellow than red ginger. The ferric reducing antioxidant power was higher ($p > 0.05$) for red than yellow ginger. It was concluded that oven dried red ginger at an inclusion level of 1.6% enhanced the growth performance of broiler chickens compared to the control and other ginger treatments and red ginger had higher total antioxidant capacity than yellow ginger in broiler feeding.

Keywords: feed additives, phytobiotics, ginger varieties, anti-oxidant capacity, broilers

EFFET DU GINGEMBRE ROUGE ET JAUNE SUR LA PERFORMANCE DE CROISSANCE ET LA CAPACITÉ ANTIOXYDANTE TOTALE DES POULETS DE CHAIR

Résumé

L'objectif de cette étude était de comparer les effets du gingembre rouge et du gingembre jaune sur la performance, la digestibilité des nutriments et la capacité antioxydante totale des poulets de chair. Dans un schéma factoriel 2 × 2 × 2 + 1, deux cent seize poulets de chair, âgés de trois semaines, ayant un poids initial de 430 g (± 0,1 g SE) ont été soumis à neuf traitements alimentaires (TRT), chaque traitement comportant 3 répétitions de 8 oiseaux par répétition. L'étude a utilisé comme facteurs deux types de gingembre traités avec deux méthodes de séchage [séchage au soleil (SS) et séchage au four (SF)] à deux niveaux d'inclusion - respectivement de 1,6% et 1,8%, et un régime témoin ne contenant aucun complément. Le Traitement I qui a servi de témoin ne contenait pas de gingembre, tandis que les traitements TRT II, III, IV et V contenaient du gingembre rouge, respectivement au taux de 1,6% (SF), 1,8% (SF), 1,6% (SS) et 1,8 (SS). Les Traitements VI à IX contenaient du gingembre jaune, respectivement au taux de 1,6% (SF), 1,8% (SF), 1,6% (SS) et de 1,8 (SS). Le type de gingembre a eu une incidence significatif sur la performance des poulets de chair, les oiseaux au traitement contenant du gingembre rouge ayant un poids corporel final plus élevé ($p < 0,05$) que les oiseaux recevant du gingembre jaune (2019 vs. 1915 g / oiseau). La digestibilité apparente des régimes pour la plupart des composants était significativement plus élevée pour le gingembre rouge par rapport au gingembre jaune. La capacité antioxydante par réduction du cuivre déterminée par la capacité antioxydante totale était significativement plus élevée pour le gingembre

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rouge par rapport au gingembre jaune, tandis que celle déterminée par le paramètre antioxydant total par piégeage de radicaux était plus élevé (p <0,05) pour le gingembre jaune par rapport au gingembre rouge. Le pouvoir antioxydant par réduction de l'ion ferrique était plus élevé (p> 0,05) pour le gingembre rouge par rapport au gingembre jaune. Il a été conclu que le gingembre rouge séché au four à un niveau d'inclusion de 1,6% a amélioré la performance de croissance des poulets de chair par rapport au traitement témoin et à d'autres traitements au gingembre, et que le gingembre rouge avait une capacité antioxydante totale plus élevée que le gingembre jaune dans l'alimentation des poulets de chair.

Mots-clés : additifs alimentaires, phytobiotique, variétés de gingembre, capacité antioxydante, poulets de chair

Introduction

Growth promoters or feed additives are primarily included in poultry feed to improve the efficiency of the bird's growth and/or laying capacity, prevent diseases and improve feed utilization. Growth-promoting antibiotics was used routinely as feed additive in animal feeds for many decades, however these were associated with antibiotic residues in the meat and eggs, leading to a ban of their prophylactic use in many countries (Diarra *et al.,* 2011). As a result, natural alternatives to antibiotics, such as herbs and medicinal plants, have attracted attention due to their wide range of potential beneficial effects (Manesh, 2012).

Zingiber officinale, a perennial plant commonly known as ginger, is widely used as a spice and treatment for certain ailments in traditional medicine and it may also act as a phytogenic feed additive (Larsen *et al.,* 1999). The active ingredients in ginger are gingerol (5%), gingerdiol (2%) and gingerdione (3%), which have the ability to stimulate digestive enzymes, affect the microbial activity and also have anti-oxidative activity (Dieumou *et al.,* 2009). Even though anti- nutrients are present in ginger, the low level at which they occur makes ginger safe for consumption by man and animals (Adanlawo and Dairo, 2007). The better performance of the broiler birds fed ginger has been attributed to improved palatability and faster digesta rate, lower transit time of feed in the digestive tract and faster gastric emptying leading to increased feed consumption (Khan *et al.,* 2012).

Poultry species under intensive management system are constantly exposed to environmental stressors such as handling stress during medications and vaccinations, social pressure, changes in ambient temperature and many other factors (Sadeghi et al. 2012). Similarly, rapid growth rate in broiler chicks may increase accumulation of body fat and increase in free radicals such reactive oxygen specie, oxidative stress and susceptibility to diseases such as heart diseases. Stress leads to the formation of free radicals, which have an undesirable effect on tissues of the body (Roberts and Sindhu, 2009). A major consequence of oxidative stress is lipid oxidation, which has been implicated for the deterioration of biologic macromolecules and impairment of physiological functions such as growth, reproduction as well as compromised immunity resulting in higher susceptibility to infectious diseases (Miller and Brzezinska-Slebodzinska, 1993). So consumption of products rich in antioxidants as additive in broiler diets may increase physiological antioxidant defenses and may decrease oxidative stress. Therefore, supplementation of synthetic antioxidants for example α-tocopheryl acetate or butylated hydroxytoluene to mitigate the oxidative stress has become a common practice in the poultry industry (Formanek *et al.,* 2001). As the toxicological safety of synthetic antioxidants has been questioned, it may be desirable to replace these conventional antioxidants with natural anti-oxidative substances (Formanek *et al.,* 2001).

Two varieties of ginger dominate ginger production in Nigeria: "Tafin-Giwa", the yellowish plump rhizomes, and "Yatsun-Biri", the red or darker smaller rhizomes (NEPC, 1999). However, only the yellow ginger has been previously extensively investigated in the diets of broiler chicken. There is also a need to investigate the effect of red ginger and as well as compare the effects of the two varieties

on performance, nutrient digestibility and total antioxidant capacity of broiler chickens, hence the need for this study.

Materials and Methods

The study was carried out at the Poultry Unit of the Teaching and Research Farm of the Obafemi Awolowo University, Ile-Ife, Osun State. Birds were obtained from a reputable hatchery at Ibadan and were reared in floor pens on wood shavings on the same diet until they were three (3) weeks old. A total of two hundred and sixteen (216) three weeks old broiler chicks were randomly assigned to nine dietary treatments of twenty-four birds per treatment in a 2×2×2+1 factorial design. The factors were two ginger types, processed by two methods of sun-drying (SD) and oven-drying (OD) at two inclusion levels of 1.6% and 1.8%. Each treatment consisted of three replicates of eight (8) birds each. Nine diets were formulated (table 1) with Treatment I as the control and contained no ginger, while TRT II, III, IV and V contained red ginger at 1.6% (OD), 1.8% (OD), 1.6% (SD) and 1.8 (SD) respectively. Treatments VI to IX contained yellow ginger at 1.6% (OD), 1.8% (OD), 1.6% (SD) and 1.8 (SD) respectively. The levels of ginger inclusion was based on the range that has been reported to promote growth in chicken and inclusion levels higher than 20g/kg diet has been reported to have adverse effect on the growth performance in chicken (Herawati, 2010; Zomrawi *et al.,* 2013). The birds were given their respective experimental diets and water ad - libitum. Body weight gain and feed intake were recorded weekly and the study lasted for five weeks. Average daily weight gain, average daily feed intake and feed conversion ratio (FCR) were calculated. For the nutrient digestibility of diets, six birds per treatment (total of 54 birds) were housed in metabolic cages in twos i.e. two birds per replicate and 3 replicates per treatment. Birds were housed for 10 days adaptation period and 5 days of collection period. The proximate composition of the experimental diets and excreta was carried out as described by AOAC (2000).

Six blood samples per treatment (two/replicate) were obtained by venipuncture of the left wing vein at 42nd day of the birds' age for total antioxidant capacity analysis. Two millilitre of blood was taken from each bird with a sterile syringe. The blood samples were collected in sterile test tubes containing EDTA (Ethylene diamine tetraacetic acid). The blood samples were centrifuged 30 minutes after collection at 1000 rpm for 15 minutes for separation of blood plasma that was used for the three antioxidant capacity analysis - total radical-trapping antioxidant parameter (TRAP), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) at the Biochemistry Laboratory of the Department of Biochemistry, Obafemi Awolowo University (OAU) Ile-Ife. The TRAP, FRAP and CUPRAC assays were carried out according to the procedures of Ghiselli et al. (1995), Benzie and Strain (1996) and Apak et al. (2004) respectively.

Data were subjected to Factorial Analysis of Variance (ANOVA) using the General Linear Model of SAS Software for Windows. Only data on ginger variety, inclusion levels and processing methods were analysed with ANOVA appropriate for factorial design, while the complete set of data including the control was analysed with one way ANOVA and the means were separated using Duncan Multiple Range Test.

Results

The proximate analysis of the experimental diets of growing broilers fed diets with red and yellow ginger is shown in table 2. The results of the proximate composition are in good agreement with the calculated values. The performance characteristics of broiler chicken fed red and yellow ginger is shown in table 3. The performance of broilers was significantly affected by ginger type with birds on red ginger having higher ($p < 0.05$) performance than birds on yellow ginger in final body weight (2016 vs. 1916 g/bird), daily weight gain (38 vs. 35 g/bird) and daily feed intake (95 vs. 91 g/bird). The performance of broilers in terms of final body weight, daily

Table 1: Gross composition of the experimental diets (%)

Ingredients	Control	Ginger 1.6%	Ginger 1.8%
Maize	52.0	54.05	54.0
Soya bean meal	26.3	25.85	25.6
Cassava flour	4.6	2.28	2.40
Wheat offal	11.15	9.77	9.75
Palm oil	1.7	2.2	2.2
Bone meal	3.5	3.5	3.5
Ginger	0	1.6	1.8
Salt	0.25	0.25	0.25
Vitamin - Mineral Premix	0.3	0.3	0.3
Methionine	0.2	0.2	0.2
Total	**100.0**	**100.0**	**100.0**
Calculated Analysis:			
Protein	18.2	18.0	18.0
ME (Kcal/kg)	3001	3007	3000
Crude fibre	3.86	3.67	3.66
Lysine	0.90	0.90	0.90
Methionine	0.30	0.30	0.30
Calcium	1.30	1.30	1.30
Phosphorus	0.70	0.70	0.70

Premix composition per kg feed:Vitamin A , 20 0,000,00 IU ,Vi t. D 3, 40,000,00IU,Vitamin E 460 mg,Vitamin K3 40 mg,Vitamin B1 60 mg,,Vitamin B2 120mg, Niacin 1,000 mg, Calcium pantothenate 200 mg,Vitamin B6 100 mg,Vitamin B12 0.5 mg, Folic acid 20 mg, Biotin 1 mg, Cholinechloride 8,000 mg, Manganese2,400 mg, Iron 2,000 mg, Zinc 1,600 mg. Copper 170 mg, Iodine 30 mg, Cobalt 6 mg, Selenium 24 mg, Anti-oxidant 2,400 mg.

Table 2: Proximate composition of the experimental diets (%)

Treatments	DM	CP	CF	EE	Ash	NFE
TRT I	89.04	18.35	3.84	4.52	9.25	54.06
TRT II	91.11	18.37	3.62	4.55	9.26	55.3
TRT III	91.17	17.9	3.62	4.25	9.37	55.98
TRT IV	89.63	18.37	3.63	4.29	9.46	53.91
TRT V	90.67	18.13	3.61	4.44	9.14	55.03
TRT VI	90.43	18.35	3.62	4.48	9.3	54.36
TRT VII	90.96	18.37	3.63	4.52	9.32	54.81
TRT VIII	89.63	18.1	3.59	4.6	9.1	54.2
TRT IX	90.27	18.37	3.73	4.26	9.28	54.62

DM – Dry matter, CP – crude protein, CF – crude fibre, EE – ether extract, ash, NFE - nitrogen free extract

weight gain and feed conversion ratio was not significantly affected by the inclusion levels of red and yellow ginger except of daily feed intake which was significantly lower in birds on 1.6% ginger inclusion level compared to birds on 1.8% ginger inclusion level (91.9 vs. 93.4 g/bird). Similarly, the processing method of ginger had no significant effect on most of the performance characteristics of broilers except on daily feed intake which was significantly lower in oven dried ginger compared to sundried ginger (92.2 vs. 93.1 g/bird). There was no significant

interaction of ginger type × inclusion level × processing method on any of the performance indices. The feed conversion ratio of all the treatments was not significantly affected by any of the imposed factors. Compared to the non-supplemented control diet TRT I, birds on TRT II had the highest final body weight (2079 g/bird) and daily weight gain (47 g/bird) than birds on other dietary treatments, while birds fed TRT VI had the least final body weight (1815 g/bird) and daily weight gain (33 g/bird).

The apparent nutrient digestibility was significantly influenced by ginger type, but there was no significant effect of processing method and inclusion levels on all digestibility values. There was also no significant interaction of ginger type × processing method × inclusion level on all digestibility values. The digestibility values for red ginger compared to yellow ginger were significantly higher for dry matter (80.0 vs. 76.7%), crude protein (74.8 vs. 68.4%), ether extract (70.8 vs. 68.7), crude fibre (62.9 vs. 52.3%) and ash (78.9 vs. 71.0%) respectively. There was no significant difference in nitrogen free extract digestibility between the red and yellow ginger varieties (89.9 vs. 89.8%).

There was no significant effect of red and yellow ginger on the FRAP values of boilers and red ginger tend to have higher FRAP values than yellow ginger (1.85 vs. 1.67 mg/ml). The TRAP values of birds fed yellow ginger were significantly higher than those of fed red ginger (0.58 vs. 0.41 mg/ml) and there was also a significant effect of inclusion level and processing method on TRAP values. Birds fed ginger at inclusion level of 1.8% had higher TRAP values than those fed 1.6% inclusion level (0.61 vs. 0.38 mg/ml). Also, birds fed sundried

Table 3. Performance characteristics of broilers chicken fed red and yellow ginger

Treatments[1]	IBW (g/bird)	FBW (g/bird)	DWG (g/bird/day)	FI (g/bird/day)	FCR
TRT I	430.60	2022.92^{ba}	45.49^{ba}	116.70^{a}	2.60
TRT II	430.80	2078.57^{a}	46.60^{a}	111.00^{e}	2.46
TRT III	430.80	2034.78^{ba}	45.82^{ba}	115.30^{b}	2.55
TRT IV	431.00	2021.74^{ba}	45.45^{ba}	113.90^{c}	2.58
TRT V	430.50	1939.58^{bac}	43.11^{bac}	112.70^{d}	2.76
TRT VI	430.80	1815.00^{c}	39.54^{c}	106.10^{i}	2.78
TRT VII	430.80	1973.91^{bac}	44.08^{bac}	109.80^{g}	2.63
TRT VIII	430.60	1997.83^{ba}	44.77^{ba}	110.00^{f}	2.49
TRT IX	430.80	1872.92^{bac}	41.20^{bac}	108.40^{h}	2.75
SEM	-	55.04	55.42	1.08	0.10
P value [2,3]	-	0.001	0.001	0.002	0.347
GV	-	0.0084	0.0122	0.0001	0.3679
IL	-	0.4981	0.5758	0.0001	0.2385
PM	-	0.6398	0.7251	0.0001	0.5907
GV*IL	-	0.2674	0.3245	0.0001	0.6006
GV*PM	-	0.1323	0.1665	0.0001	0.1170
IL*PM	-	0.0514	0.0415	0.0001	1.1314
GV*IL*PM	-	0.0991	0.1260	0.8020	0.3250

[1]IBW = initial body weight, FBW = final body weight, DWG = daily weight gain, FI = feed intake, FCR = feed conversion ratio, SEM = standard error of mean,

*[2]GV = ginger variety, *IL = inclusion level, *PM = processing method, GV*IL = two way interaction between ginger variety and inclusion level, GV*PM = two way interaction between ginger variety and processing method, IL*PM = two way interaction between inclusion level and processing method, GV*IL*PM = three way interaction between ginger variety, inclusion level and processing method*

[3]Means within each column with different superscripts are significantly different (P< 0.05).

Table 4: Apparent digestibility broilers fed red and yellow ginger varieties (%)

Treatments	Dry matter	Crude protein	Crude fibre	EE1	Ash	NFE1
TRT I	83.41[a]	78.13[a]	72.57[a]	76.31[a]	81.17[a]	90.55
TRT II	81.52[ab]	76.95[ab]	62.26[bc]	78.20[a]	83.69[a]	90.04
TRT III	81.32[ab]	74.73[ab]	66.73[ab]	77.83[a]	80.38[a]	90.52
TRT IV	79.20[bc]	75.05[ab]	55.63[cd]	64.02[b]	79.72[ab]	89.31
TRT V	78.11[bc]	72.29[bc]	66.95[ab]	63.33[b]	71.99[cd]	89.57
TRT VI	76.88[c]	66.27[d]	64.36[abc]	54.88[c]	68.61[cd]	91.51
TRT VII	77.89[bc]	69.11[cd]	45.64[e]	73.72[a]	67.20[d]	91.79
TRT VIII	75.90[c]	66.21[d]	51.13[de]	73.43[a]	74.15[bc]	88.31
TRT IX	76.27[c]	72.16[bc]	47.95[de]	72.93[a]	74.10[bc]	87.65
SEM	0.94	1.25	2.79	1.72	1.60	0.64
P value	0.002	0.001	<0.001	0.001	<0.001	0.0604
GV	0.001	<0.001	<0.001	0.205	<0.001	0.947
IL	0.029	0.769	0.050	0.106	0.990	0.004
PM	0.977	0.420	0.467	0.015	0.037	0.895
GV * IL	0.401	0.129	0.589	0.000	0.000	0.048
GV * PM	0.442	0.008	0.000	0.008	0.100	0.677
IL * PM	0.660	0.583	0.015	0.007	0.587	0.670
GV * IL * PM	0.939	0.438	0.303	0.009	0.308	0.790

[1]EE – ether extract, ash, NFE - nitrogen free extract

*[2]GV = ginger variety, *IL = inclusion level, *PM = processing method, GV*IL = two way interaction between ginger variety and inclusion level, GV*PM = two way interaction between ginger variety and processing method, IL*PM = two way interaction between inclusion level and processing method, GV*IL*PM = three way interaction between ginger variety, inclusion level and processing method*

[3]Means within each column with different superscripts are significantly different (P< 0.05).

ginger had significantly higher TRAP values than those fed oven dried ginger (0.56 vs. 0.42 mg/ml). There was also a significant interaction of ginger variety × inclusion level × processing method on TRAP values. There was no significant effect of inclusion level on CUPRAC values of birds fed red and yellow gingers but there were significant effect on ginger type and processing method on the CUPRAC values of birds. Birds fed red ginger had higher ($p < 0.05$) CUPRAC values than those fed yellow ginger (1.86 vs. 1.58 mg/ml). Also, birds fed oven dried ginger had higher ($p < 0.05$) CUPRAC values than those fed sundried ginger (1.82 vs. 1.61 mg/ml). Compared to the non-supplemented control treatment I, the highest ($p < 0.05$) TRAP value was recorded by broilers fed sundried yellow ginger at 1.8% inclusion level , while highest ($p < 0.05$) cupric ion (Cu2+) reducing ability was recorded by broilers fed oven dried red ginger at 1.8% inclusion level.

Discussion

The performance of broilers was significantly affected by ginger type with birds on red ginger having significantly higher final body weight, daily weight gain and daily feed intake than birds on yellow ginger. The 1.6% inclusion of red ginger which resulted in the best performance in the present study is similar to the 1.5% ginger inclusion level of Herawati (2010), who reported a significantly higher value in final body weight of broilers, fed red ginger at 1.5%. Herawati (2010) fed graded levels of ginger at 0, 0.5, 1.0, 1.5 and 2% ginger and reported that broilers fed 1.5% ginger had the significantly highest daily weight gain and feed intake was significantly depressed when bird were fed higher ginger level of 2.0%. However, feed conversion ratio was not significantly affected by ginger supplementation over the range of intake of 0 - 2 %, which is

similar to our observation in the present study in which the different inclusion levels of red and yellow ginger had no effect on the feed conversion ratio of broiler chicken. Similarly, Ademola et al. (2009) fed supplemental ginger to finishing broiler chicken at 1.0, 1.5 and 2.0% and observed that the inclusion levels of ginger at 2% depressed final body weight and also had a negative effect on feed conversion ratio which was significant higher than the non-supplemented control. However, the lower inclusion levels of ginger of 1.0 and 1.5% were not different from the non-supplemented control in terms daily weight gain, feed intake and feed conversion ratio. This is similar to the result in the present study, in which the highest performance in terms of final body weight and daily weight gain on 1.6% oven dried ginger inclusion level was only a numerical increase over the non-supplemented control. However, improved performance at lower concentration of supplemental ginger in terms of final body weight, daily weight gain and feed conversion ratio at 0.25% ginger inclusion level compared to non-supplemented control was reported Onu (2010) in a growth trial of 5-10 weeks old with broiler chicken. Also Selim et al. (2013) reported an improvement in the performance of broiler chicken in terms of final body weight, weight gain and feed conversion ratio at 0.5 and 1.0% supplementation of ginger aqueous extract in a 40 days study compared to non-supplemental control. In other studies in laying hen, the essential oil of ginger has been reported not to have any significant effect on the feed conversion ratio of laying birds (Nasiroleslami and Torki, 2010; Akbarian *et al.,* 2011; Zhao *et al.,* 2011). The apparent digestibility values are in good agreement with the growth performance data and followed a similar trend and red ginger had higher digestibility values for most proximate constituents except for nitrogen free extract digestibility.

Table 5: Antioxidant activity in broilers fed red ginger and yellow ginger (mg/ml).

Treatments	FRAP	TRAP	CUPRAC
TRT I	1.833	0.245^{ef}	1.826^{ab}
TRT II	2.026	0.332^{cde}	1.948^{a}
TRT III	1.688	0.592^{b}	1.748^{ab}
TRT IV	1.938	0.420^{c}	1.973^{a}
TRT V	1.752	0.281^{def}	1.764^{ab}
TRT VI	1.706	0.339^{cd}	1.723^{ab}
TRT VII	1.678	0.235^{f}	1.565^{bc}
TRT VIII	1.731	0.596^{b}	1.674^{b}
TRT IX	1.555	1.142^{a}	1.365^{c}
Pooled SEM	0.0381	0.0661	0.0468
P value	0.048	< 0.001	< 0.001
GV	0.011	< 0.001	< 0.001
IL	0.601	< 0.001	0.328
PM	0.012	< 0.001	0.002
GV * IL	0.750	< 0.001	0.184
GV * PM	0.191	0.003	0.779
IL * PM	0.986	0.011	0.446
GV * IL * PM	0.218	< 0.001	0.497

*[1]GV = ginger variety, *IL = inclusion level, *PM = processing method, GV*IL = two way interaction between ginger variety and inclusion level, GV*PM = two way interaction between ginger variety and processing method, IL*PM = two way interaction between inclusion level and processing method, GV*IL*PM = three way interaction between ginger variety, inclusion level and processing method*

[2]Means within each column with different superscripts are significantly different (P< 0.05).

In the present study, the total antioxidant capacity of the plasma in broilers fed ginger were measured with three methods namely, total radical-trapping antioxidant parameter (TRAP), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC). Ferric reducing antioxidant power measures the antioxidant power of food based on the chelating capacity of ferrous ion from Fe3+ to Fe2+ (Sohaib *et al.,* 2015). Total radical-trapping antioxidant parameter (TRAP) is the quantitative measure of the total secondary antioxidant content of a biological fluid using known quantity of peroxyl radicals (Sies, 2007), while the cupric ion reducing antioxidant capacity is also the measurement of the total plasma antioxidant capacity using copper II neocuproine reagent as the chromogenic oxidizing agent (Apak *et al.,* 2008). The total antioxidant capacity of measured by FRAP in the plasma of broiler chicken was not significantly affected by the supplementation of red and yellow ginger, although the FRAP value of birds fed oven dried red ginger at 1.6% inclusion level tended to be higher than that of the non-supplemented control. Although in an earlier study on broiler, the total antioxidant capacity measured by FRAP was significant improved at a ginger inclusion level of 0.75% (Sadeghi *et al.,* 2012). Sadeghi et al. (2012) fed ginger root to broilers challenged and unchallenged with Salmonella enteritidis by oral gavage at four levels of 0, 0.25, 0.50 and 0.75%. Challenged and unchallenged broilers fed diets supplemented with ginger had significantly higher FRAP values than the non supplemented challenged and unchallenged control. The TRAP values of birds fed yellow ginger were significantly higher than those of fed red ginger and birds fed ginger at inclusion level of 1.8% had higher TRAP values than those fed 1.6% inclusion level. Birds fed red ginger had higher CUPRAC values than those fed yellow ginger. Also, birds fed oven dried ginger had higher CUPRAC values than those fed sundried ginger. Other studies using other antioxidant indices have also demonstrated the anti-oxidative properties of ginger in broiler chicken (Selim *et al.,* 2013; Zhang *et al.,* 2009). Zhang et al. (2009) reported that supplementation of ginger root powder at 0.5% significantly increased glutathione peroxidase at day 21 and total superoxide dismutase at day 42 in broiler chicken fed ginger processed to different particle sizes. Similarly, previous in vitro study by Krishnakanta and Lokesh (1993) in comparing the antioxidative properties of ginger (zingerone), coriander (linalool), pepper (piperine) and tumeric extract showed the ginger and coriander extracts showed anti-oxidative properties, while pepper and tumeric extract showed no anti-oxidative properties. The responses of broiler chicken to ginger inclusion in their diets have been varied in literature and this has been attributed to varieties of ginger used, their processing method, dose and the duration of the experiments.

Conclusion

It was concluded that oven dried red ginger at an inclusion level of 1.6% enhanced the growth performance of broiler chickens compared to the control and other ginger treatments and red ginger had higher total antioxidant capacity than yellow ginger in broiler feeding.

References

Ademola S. G., Farinu G. O., Babatunde G. M., 2009. Serum lipid, growth and haematological parameters of broilers fed garlic, ginger and their mixtures. World Journal of Agricultural Sciences 5: 99-104

Adanlawo I. G., Dairo F.A. S., 2007. Nutrient and anti-nutrient constituents of ginger (Zingiber officinale, Roscoe) and the influence of its ethanolic extract on some serum enzymes in albino rats. International J. of Biological Chemistry (1): 38- 46.

Akbarian A, Abolghasem G, Ahmadi S, Hossein M., 2011. Effects of ginger root (Zingiber officinale) on egg yolk cholesterol, antioxidant status and performance of laying hens. Journal of Applied Animal Science 39: 19-21.

AOAC, 2000. Official Methods of Analysis of the Association of official Analytical Chemist Published by the Association of analytical chemists, Washington DC.

Apak, R., Guclu, K., Mustafa, O., Karademir, S., 2004. Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method, Agric. Food Chem., 52 (26), p 7970–7981.

Benzie, I. F., Strain, J. J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power: the FRAP assay. Analytical Biochemistry, 239(1): 70-76.

Diarra S. S., Kwari I. D., Girgiri, Y. A., Saleh B., Igwebuike J. U., 2011. The use of sorrel (Hibiscuss sabdariffa) seed as a feed ingredient for poultry: A review. Research Opinions in Animal & Veterinary Sciences 1: 573-577.

Dieumou, F. E., Teguia A., Kuiate J. R., Tamokou J. D., Fonge N. B., Dongmo M.C., 2009. Effects of ginger (Zingiber officinale) and garlic (Allium sativum) essential oils on growth performance and gut microbial population of broiler chickens. Livest. Res. for Rural Dev., 21: 25-34

Formanek, Z., Kerry, J. P., Higgins, F. M., Buckley, D. J., Morrissey, P.A., Farkas, J., 2001. Addition of synthetic and natural antioxidants to α-tocopheryl acetate supplemented beef patties: effect of antioxidants and packaging on lipid oxidation. Meat Science 58, 337–341.

Ghiselli, A., Serafini, M., Maiani, G., Assini, E., Ferro-Luzzi, A., 1995. A fluorescence-based method for measuring total plasma antioxidant capability. Free Rad Biol Med; 18: 29-36.

Herawati, 2010. Effect of feeding red ginger as phytobiotic on body weight gain, feed conversion and internal organs condition of broiler. International Journal of Poultry Science 9 (10): 963-967.

Khan, M., Qureshi S., Naz S., Nikousefat Z., Laudadio V., 2012. Potential applications of ginger (Zingiber officinale) in poultry diets. World's Poultry Science Journal, Vol. 68; 245 – 252.

Larsen K., Ibrahim H., Khaw S. H. and Saw L. G., 1999. Gingers of Peninsular Malaysia and Singapore. Natural History Publications (Borneo), Kota Kinabalu, Malaysia.

Manesh M. K., 2012. Influence of poly germander (Teucrium polium) on performance of male broilers. Research Opinions in Animal & Veterinary Sciences 2: 69-71.

Miller, J. K., Brzezinska-Slebodzinska E., 1993. Oxidative stress, antioxidants and animal function J Dairy of Science 76, 2812–2823.

Nasiroleslami M., Torki M, 2010. Including Essential Oils of Fennel (Foeniculum Vulgare) and Ginger (Zingiber Officinale) to Diet and Evaluating Performance of Laying Hens. Advances in Environmental Biology 4: 341-345.

NEPC, 1999. Product profile of ginger. Nigerian export promotion council B/K 312, Kumba st, Wuse, Zone II, Abuja, pp. 1-6.

Onu P. N., 2010. Evaluation of two herbal spices as feed additives for finisher broilers. Biotechnology in Animal Husbandry 26: 383-392.
Roberts, C. K., Sindhu K. K., 2009. Oxidative stress and metabolic syndrome. Life Science, 84: 705-712.

Sadeghi A. A., Izadi W., Shawrang P., Chamani M., Aminafsha M., 2012. Effect of Ginger (Zingiber officinale) Powder Supplementation on Total Antioxidant Capacity of Plasma and Oxidative Stress in Broiler Chicks Challenged with Salmonella enteritidis. World Applied Sciences Journal 18 (1): 130-134

Selim N. A., Youssef S. F., Abdel-Salam A. F., Nada S. A., 2013. Evaluation of Some Natural Antioxidant Sources in Broiler Diets: 1-Effect on Growth, Physiological, Microbiological and Immunological Performance of Broiler Chicks. International Journal of Poultry Science 12 (10): 561-571

Sies H. (2007). Total Antioxidant Capacity: Appraisal of a Concept. J. Nutr. 137: 1493-1495

Sohaib M., M. S. Butt, M. A. Shabbir and M. Shahid (2015). Lipid stability, antioxidant potential and fatty acid composition of broilers breast meat as influenced by quercetin in combination with α-tocopherol enriched diets Lipids in Health and Disease (2015) 14:61

Zhang, G. F., Yang, Z. B., Wang, Y., Yang, W. R., Jiang, S. Z. And Gai, G. S. (2009). Effects of ginger root (Zingiber officinale) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. Poultry Science 88:2159-2166.

Zhao X, Yang ZB, Yang WR, Wang Y, Jiang SZ. and Zhang GG, 2011. Effects of ginger root (Zingiber officinale) on laying performance and antioxidant status of laying hens and on dietary oxidation stability. Poultry Science 90: 1720 - 1727.

Zomrawi WB., A Abdel Attika, BM. Dousa and Mahala AG, 2013. The Effect of Dietary Ginger Root Powder (Zingiber officinale) on Broiler Chicks Performance, Carcass Characteristic and Serum Constituents. Journal of Animal Science Adv 3(2): 42 - 47.

GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF EXOTIC TURKEY BROILERS FED VARYING LEVELS OF ENERGY TO PROTEIN (E/P) RATIOS

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Abstract

The objective of the study was to determine the energy/protein (E/P) ratio of exotic turkeys in the humid tropical environment of Nigeria. In a 16 weeks feeding trial, a total of 144 day-old exotic poults with initial weight of 57g (± 0.4 SE) were randomly assigned to six dietary treatments in a 2×3 factorial design with 24 poults per treatment and each treatment was replicated twice. The factors were two levels of metabolisable energy (ME; kcal/kg) and 3 levels of crude protein (CP). At 0-4 weeks, diets 1-3 and 4-6 contained 2800 and 2900 ME and each with 30, 28 and 26% CP and E/P ratio of diets 1-6 were 93:1, 100:1, 107:1, 96:1, 104:1 and 112:1 respectively. At 4 - 8 weeks, diets 1 - 3 and 4 - 6 comprised energy levels of 2900 and 3000 ME each with CP of 28, 26, and 24% respectively and E/P ratio of diets 1-6 were 104:1, 116:1, 112:1, 107:1, 115:1 and 125:1 respectively. At 8 – 12 weeks, diets 1 - 3 and 4 - 6 also comprised of energy levels 3000 and 3100 ME respectively with 24, 22 and 20% CP and E/P ratio of diets 1-6 were 125:1, 136:1, 150:1, 129:1, 141:1 and 155:1 respectively. At weeks 12 - 16, diets 1 - 3 and 4 – 6 contained 3000 and 3100 ME at CP levels of 21, 19 and 17% and the E/P ratio of diets 1-6 were 143:1, 156:1, 176:1, 148:1, 163:1 and 182:1 respectively. There was no significant effect of energy, protein or their interaction on any of the performance characteristics of poults from 0-16 weeks of age. There was no significant effect of energy, protein or E/P ratio on any of the proximate digestibility values. It was concluded that the E/P ratio, energy and protein requirements of exotic turkey may not be more than 107:1 (2800 ME and 26% CP), 121:1 (2900 ME and 24% CP), 150:1 (3000 ME and 20% CP) and 177:1 (3000 ME and 17% CP) for 0-4, 4-8, 8-12 and 12-16 weeks, respectively.

Keywords: nutrient requirements, energy/protein ratio, poults, protein, energy

PERFORMANCE DE CROISSANCE ET DIGESTIBILITÉ DES NUTRIMENTS DES DINDES DE CHAIR EXOTIQUES NOURRIS AVEC DIFFÉRENTS NIVEAUX DE RATIOS ÉNERGIE/PROTÉINES

Résumé

L'objectif de l'étude était de déterminer le ratio énergie / protéines (E / P) de dindes exotiques dans un environnement tropical humide au Nigeria. Dans un essai alimentaire de 16 semaines, 144 dindonneaux exotiques au total, âgés d'un jour, avec un poids initial de 57 g (± 0,4 SE) ont été répartis de manière aléatoire à six traitements alimentaires dans un schéma factoriel 2 × 3 avec 24 dindonneaux par traitement ; et chaque traitement a été répété deux fois. Les facteurs utilisés étaient deux niveaux d'énergie métabolisable (EM; kcal / kg) et 3 niveaux de protéines brutes (PB). A 0-4 semaines, les régimes alimentaires 1-3 et 4-6 contenaient respectivement 2800 et 2900 EM, chacun avec 30, 28 et 26% PB, et les ratios E/P des régimes 1-6 étaient respectivement de 93 :1 ; 100: 1 ; 107: 1 ; 96: 1 ; 104: 1 et 112: 1. À 4 - 8 semaines, les régimes 1-3 et 4-6 comprenaient des niveaux d'énergie de 2900 à 3000 EM chacun, avec des taux respectifs de PB de 28, 26, et 24%, tandis que les ratios E / P des régimes 1-6 étaient respectivement de 104: 1 ; 116 : 1 ; 112: 1 ; 107: 1 ; 115: 1 et 125: 1. À 8 - 12 semaines, les régimes alimentaires 1-3 et 4-6 comprenaient également des niveaux d'énergie de 3000 et 3100 EM, respectivement avec 24, 22 et 20% de PB, tandis que les ratios E / P des régimes 1-6 étaient respectivement de 125: 1 ; 136: 1 ; 150: 1 ; 129: 1 ; 141: 1 et 155: 1. Aux semaines 12 à 16, les régimes alimentaires 1-3 et 4-6 contenaient 3,000 et 3,100 EM avec des niveaux de PB de 21, 19 et 17% ; et les ratios E / P des régimes 1-6 étaient respectivement de 143: 1 ; 156: 1 ; 176 : 1

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; 148: 1 ; 163: 1 et 182: 1. On n'a pas noté d'effet significatif de l'énergie, des protéines ou de leur interaction sur l'une des caractéristiques de performance des dindonneaux âgés de 0-16 semaines. Il n'y a pas eu d'effet significatif de l'énergie, des protéines ou du ratio E / P sur l'une quelconque des valeurs de digestibilité. Il a été conclu que les ratios E / P, les besoins en énergie et protéines des dindes exotiques ne peuvent pas être plus de 107: 1 (2800 EM et 26% PB) ; 121: 1 (2900 EM et 24% PB) ; 150: 1 (3000 EM et 20% PB) et 177: 1 (3000 EM et 17% PB) respectivement pour les oiseaux de 0-4, 4-8, 8-12 et 12-16 semaines.

Mots-clés : Besoins en nutriments, ratio énergie/protéines, dindonneaux, protéine, énergie

Introduction

Mixed rations for farm animals are usually formulated with careful attention to metabolisable energy, protein and other nutrients. The energy and protein components of feed constitute the largest fractions of poultry diets and the productivity of poultry is partly regulated by the energy to protein ratio of the diet (Oyedeji and Atteh, 2005; Peñaranda-Ali *et al.,* 2010). The dietary energy to protein ratio is an important index in poultry nutrition for optimum growth performance and carcass quality, and also to promote lean meat deposition (Waldroup *et al.,* 2003; Matanmi *et al.,* 2014). Energy in excess of need for protein deposition in growing animals is usually converted to fat and stored in fat deposits especially in the skin, cloaca and abdominal regions in poultry and this also reduces feed efficiency and the quality of poultry product from birds fed excessive dietary energy. Similarly, excessive protein intake in birds is both economically and physiologically expensive, because protein cost is a major cost in feed formulation for poultry and the excretion of excess nitrogen through deamination also has physiological energy cost. The high cost of protein supplement/concentrate and the need to reduce nitrogen excretion from animal manure into the environment are other important considerations in precision ration formulation for farm animals (Peñaranda-Ali *et al.,* 2010).

National Research Council (1994) reported energy/protein ratio of turkey as 100:1 (2800 kcal metabolisable energy/kg diet and 28% crude protein), 112:1 (2900 metabolisable energy/kg diet and 26% crude protein), 136:1 (3000 metabolisable energy/kg diet and 22% crude protein) and 163:1 (3100 metabolisable energy/kg diet and 19% crude protein) for 0-4 weeks, 4-8 weeks, 8-12 weeks and 12-16 weeks respectively for large white turkeys reared in the temperate region, while Olomu (1995) also reported an energy/protein ratio of 93:1 (2800 kcal metabolisable energy/kg diet and 30% crude protein) and 122:1 (2800 kcal metabolisable energy/kg diet and 23% crude protein) for turkeys from 0 to 8 weeks and 8 to 16 weeks of age. To our knowledge, current estimates for energy and protein requirements/ratio are lacking for turkeys in humid tropical environments of Nigeria. Therefore, this study was carried out to determine the energy, protein and energy/protein ratio of exotic turkeys in the humid tropical environment of Nigeria.

Materials and Methods

The experiment was carried out at the Turkey Unit of the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife. A total of 144 day-old exotic poults of Nicholas strain were randomly assigned to six dietary treatments in a floor pen on wood shavings in a 2×3 factorial design with 24 poults per treatment and two replicates of 12 birds each. The dimension of each floor pen was approximately 14 m2. Birds were brooded with electricity using incandescent bulbs and supplemental heat was provided on cold nights using heat produced from burning charcoal in pots. The factors were two levels of metabolisable energy and three levels of dietary crude protein. The gross composition of the experimental diets is presented in table 1 (0-4 weeks and 4-8 weeks) and table 2 (8-12 weeks and 12-16 weeks) and the study lasted for 16 weeks. At the phase one of the study (0-4 weeks), Diets 1-3 contained 2800 kcal/kg ME at

30, 28 and 26% crude protein (CP) levels, while diets 4-6 contained 2900 kcal/kg ME at 30, 28 and 26% CP levels. The corresponding energy to protein (E/P) ratio of diets 1-6 were 93:1, 100:1, 107:1, 96:1, 104:1 and 112:1 respectively. At 4 - 8 weeks (phase 2), TRT 1 - 3 and 4 - 6 comprised energy levels of 2900 and 3000 kcal/kg ME each with crude protein levels of 28, 26, and 24% respectively. The energy/protein ratio of diets 1-6 at this phase were 104:1, 116:1, 112:1, 107:1, 115:1 and 125:1 respectively. At 8 – 12 weeks (phase 3), TRT 1 - 3 and 4 - 6 also comprised of energy levels 3000 and 3100 kcal/kg ME respectively with 24, 22 and 20% CP inclusion levels. The energy/protein ratio of diets 1-6 at this phase were 125:1, 136:1, 150:1, 129:1, 141:1 and 155:1 respectively. At week 12 – 16 (phase 4), poults were fed diets containing the same energy levels kept at 3000 and 3100 kcal/kg ME respectively at the three protein levels of 21, 19 and 17%. The energy/protein ratio of diet 1-6 at this phase were 143:1, 156:1, 176:1, 148:1, 163:1 and 182:1 respectively. Water and feed were supplied ad libitum. Data on body weight changes and feed intake were recorded fortnightly and weekly respectively. Daily weight gain, daily feed intake and feed conversion ratio (FCR) were calculated. For digestibility study, two birds were selected from each treatment at the end

Table 1: Gross composition of the experimental diets (0 - 4; 4 - 8 weeks)

0-4 weeks	**TRT I**	**TRT II**	**TRT III**	**TRT IV**	**TRT V**	**TRT VI**
ME (kcal/kg)	2800	2800	2800	2900	2900	2900
Crude protein level (%)	30	28	26	30	280	26
Energy/protein ratio (E/P)	93:1	100:1	107:1	97:1	104:1	112:1
Ingredients						
Maize	35.70	34.50	38.00	30.00	37.00	38.00
Soya bean meal	54.30	49.80	43.80	55.00	50.50	43.00
Wheat Offal	2.30	7.96	10.25	4.47	3.96	9.09
Fish Meal (72% CP)	3.00	2.00	2.00	3.00	2.00	2.56
Palm Oil	-	1.00	1.00	2.80	1.80	2.40
Bone Meal	3.60	3.60	3.60	3.60	3.60	3.60
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.10	0.14	0.18	0.13	0.14	0.18
Lysine	-	-	0.17	-	-	0.17
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values						
ME (kcal /kg)	2821	2815	2816	2909	2909	2901
Crude protein	30.01	28.16	26.25	30.12	28.03	26.11
Crude fibre	4.47	4.66	4.54	4.64	4.42	4.40
Methionine	0.54	0.55	0.57	0.57	0.55	0.57
Lysine	1.77	1.64	1.66	1.79	1.63	1.66
Calcium	1.59	1.53	1.52	1.60	1.53	1.55
Phosphorus	1.05	1.01	1.29	1.06	1.01	0.99
4 - 8 weeks						
ME (kcal/kg)	2900	2900	2900	3000	3000	3000
Protein level	28	26	24	28	26	24

	TRT I	TRT II	TRT III	TRT IV	TRT V	TRT VI
Energy/protein ratio (E/P)	104:1	116:1	112:1	107:1	115:1	125:1
Ingredients						
Maize	38.75	42.24	47.90	43.01	48.11	51.54
Soybean meal	47.22	41.00	34.60	48.63	42.40	36.10
Wheat offal	7.00	9.68	10.73	1.01	2.40	5.09
Fish meal (72%)	3.00	3.00	3.00	3.00	3.00	3.00
Palm oil	1.00	1.00	0.50	1.32	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Premix	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.03	0.05	0.08	0.03	0.05	0.08
Lysine	-	0.03	0.19	-	0.04	0.19
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values						
ME (kcal /kg)	2902	2904	2905	3000	3008	3006
Crude protein	28.00	26.07	24.00	28.03	26.04	24.06
Crude fibre	4.47	4.36	4.36	4.15	3.95	3.84
Methionine	0.45	0.45	0.45	0.45	0.45	0.45
Lysine	1.63	1.50	1.50	1.61	1.50	1.50
Calcium	1.01	1.00	0.99	1.01	1.00	0.99
Phosphorus	0.76	0.74	0.71	0.76	0.73	0.70

Table 2: Gross composition of the experimental diets (8 - 12; 12 - 16 weeks)

8-12 weeks	TRT I	TRT II	TRT III	TRT IV	TRT V	TRT VI
ME (kcal/kg)	3000	3000	3000	3100	3100	3100
Crude protein level (%)	24	22	20	24	22	20
Energy/protein ratio (E/P)	125:1	136:1	150:1	129:1	141:1	155:1
Ingredients						
Maize	51.09	54.40	59.96	54.24	56.54	61.90
Soya bean meal	35.90	31.60	25.10	37.05	32.80	26.24
Wheat Offal	6.00	7.83	9.08	1.00	3.59	5.00
Fish Meal (72% CP)	3.00	2.00	2.00	3.00	2.00	2.00
Palm Oil	1.00	1.00	0.50	1.70	1.90	1.50
Bone Meal	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin-mineral premix	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.05	0.05	0.05	0.05	0.05	0.05
Methionine	0.01	0.04	0.07	0.01	0.04	0.07
Lysine	-	0.13	0.29	-	0.13	0.29
Total	100.00	100.00	100.00	100.00	100.00	100.00

Calculated values	**TRT I**	**TRT II**	**TRT III**	**TRT IV**	**TRT V**	**TRT VI**
ME (kcal /kg)	3002	3005	3004	3103	3104	3105
Crude protein	24.09	22.12	20.02	24.06	22.14	20.03
Crude fibre	3.90	3.83	3.62	3.61	3.59	3.39
Methionine	0.38	0.38	0.38	0.38	0.38	0.38
Lysine	1.32	1.30	1.30	1.32	1.30	1.30
Calcium	0.99	0.92	0.91	0.98	0.92	0.91
Phosphorus	0.70	0.65	0.62	0.70	0.65	0.62
12 - 16 weeks						
ME (kcal/kg)	3000	3000	3000	3100	3100	3100
Protein level	21	19	17	21	19	17
Energy/protein ratio (E/P)	143:1	156:1	176:1	148:1	163:1	182:1
Ingredients						
Maize	56.80	59.00	63.74	58.70	61.00	64.74
Soybean meal	27.00	23.00	17.20	28.10	24.00	18.20
Wheat offal	9.70	12.44	13.80	5.70	8.44	10.80
Fish meal (72%)	2.50	1.50	1.00	2.50	1.50	1.00
Palm oil	1.00	1.00	1.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Premix	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.05	0.05	0.05	0.05	0.05	0.05
Methionine	-	0.03	0.06	-	0.03	0.06
Lysine	-	0.03	0.20	-	0.03	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values						
ME (kcal /kg)	3012	3013	3020	3113	3103	3105
Crude protein	21.01	19.21	17.01	21.00	19.17	17.04
Crude fibre	3.74	3.75	3.57	3.51	3.51	3.41
Methionine	0.33	0.33	0.33	0.33	0.33	0.33
Lysine	1.10	1.00	1.00	1.10	1.00	1.00
Calcium	0.94	0.88	0.84	0.94	0.87	0.83
Phosphorus	0.65	0.61	0.56	0.65	0.60	0.56

Table 3: Proximate Composition of Turkey diets (0 - 16 weeks)

0-4 weeks	**TRT I**	**TRT II**	**TRT III**	**TRT IV**	**TRT V**	**TRT VI**
Calculated						
Energy (kcal ME/kg)	2800	2800	2800	2900	2900	2900
Crude protein (%)	30	28	26	30	28	26
Energy/protein ratio	93:1	100:1	108:1	97:1	104:1	112:1
Analysed						
Dry matter (%)	89.25	89.63	90.10	89.80	88.89	89.39
Crude protein (%)	29.53	27.56	26.28	29.97	28.44	26.25
Crude fiber (%)	4.07	4.62	4.11	4.61	5.88	4.49
Ether extract (%)	2.07	2.09	3.49	2.62	2.01	2.59
Ash (%)	8.01	9.82	9.74	10.87	9.25	8.71
NFE (%)	45.58	45.54	46.55	41.73	45.33	47.34
4-8 weeks						
Calculated						
Energy (kcal ME/kg)	2900	2900	2900	3000	3000	3000
Crude protein (%)	28	26	24	28	26	24
Energy/protein ratio	104:1	116:1	112:1	107:1	115:1	125:1
Analysed						
Dry matter (%)	88.25	86.13	88.87	89.55	89.84	89.46
Crude protein (%)	27.50	26.25	23.59	27.90	25.59	24.41
Crude fiber (%)	3.76	4.70	3.68	4.22	4.00	4.38
Ether extract (%)	3.30	2.41	2.59	3.54	3.73	3.45
Ash (%)	9.12	9.60	7.44	9.86	7.62	6.66
NFE (%)	44.51	42.73	51.53	43.83	48.89	50.69
8-12 weeks						
Calculated						
Energy (kcal ME/kg)	3000	3000	3000	3100	3100	3100
Crude protein (%)	24	22	20	24	22	20
Energy/protein ratio	125:	136:1	150:1	129:1	141:1	155:1
Analysed						
Dry matter (%)	89.04	89.55	89.91	88.12	88.98	88.94
Crude protein (%)	24.49	22.09	19.53	23.97	21.55	19.59
Crude fiber (%)	2.91	4.46	3.90	3.67	2.77	3.04
Ether extract (%)	1.59	2.26	1.49	1.45	2.06	1.66
Ash (%)	8.95	9.37	7.47	7.23	8.48	8.77
NFE (%)	51.09	51.37	57.80	52.59	54.29	56.88
12-16 weeks						
Calculated						
Energy (kcal ME/kg)	3000	3000	3000	3100	3100	3100
Crude protein (%)	21	19	17	21	19	17
Energy/protein ratio	143:1	156:1	176:1	148:1	163:1	182:1

Analysed	TRT I	TRT II	TRT III	TRT IV	TRT V	TRT VI
Dry matter (%)	88.85	88.78	89.38	90.07	89.61	89.64
Crude protein (%)	21.17	19.25	16.84	21.00	19.03	17.28
Crude fiber (%)	4.06	4.38	4.08	4.72	4.32	3.75
Ether extract (%)	2.10	2.26	2.72	2.01	2.06	2.45
Ash (%)	9.31	8.67	9.72	8.79	7.09	10.49
NFE (%)	52.40	54.23	56.01	53.54	57.12	55.68

Table 4: Growth performance of turkeys fed varying energy/protein ratio (0-16 weeks)

Treatments	Initial body weight (g/bird)	Final body weight (g/bird)	Daily weight gain (g/bird)	Daily Feed Intake (g/bird)	Feed conversion ratio g/g
0- 4 weeks					
TRT I	58.01	713.21	23.44	31.23	1.33
TRT II	57.95	671.31	21.94	24.10	1.10
TRT III	55.99	663.81	21.71	25.96	1.20
TRT IV	56.06	626.03	20.36	26.72	1.31
TRT V	56.38	572.56	18.44	24.56	1.33
TRT VI	56.03	624.10	20.29	25.93	1.28
SEM	0.375	0.694	0.687	0.991	0.033
P values					
Energy	0.150	0.097	0.009	0.082	0.350
Protein	0.406	0.620	0.615	0.340	0.528
Energy × Protein	0.520	0.807	0.815	0.585	0.458
4-8 weeks					
TRT I	713.21	2373.8	59.31ba	136.01ba	2.29
TRT II	671.31	2226.7	55.53 b	127.80 b	2.30
TRT III	663.81	2305.0	58.61ba	128.11 b	2.19
TRT IV	626.03	2481.4	66.26ba	131.87b	1.99
TRT V	572.56	2677	75.16a	141.60a	1.88
TRT VI	624.10	2540.5	68.44ba	134.10b	1.95
SEM	0.694	55.588	0.687	0.991	0.031
P values					
Energy	0.097	0.023	0.025	0.045	0.044
Protein	0.620	0.958	0.973	0.247	0.545
Energy × Protein	0.807	0.334	0.553	0.039	0.817
8-12 weeks					
TRT I	2373.8	5316.70	105.10	253.00	2.41
TRT II	2226.7	4989.70	98.68	225.87	2.31
TRT III	2305.0	5200.00	103.39	252.9	2.45
TRT IV	2481.4	5440.90	105.70	253.72	2.40
TRT V	2677	5415.00	97.75	261.79	2.68

Treatments	Initial body weight (g/bird)	Final body weight (g/bird)	Daily weight gain (g/bird)	Daily Feed Intake (g/bird)	Feed conversion ratio g/g
TRT VI	2540.5	5640.90	110.73	254.48	2.30
SEM	55.588	131.60	4.744	2.279	0.054
P values					
Energy	0.023	0.043	0.267	0.435	0.289
Protein	0.958	0.175	0.175	0.732	0.175
Energy × Protein	0.334	0.310	0.311	0.444	0.580
12-16 weeks					
TRT I	5316.70	7691.70	84.82	219.12	2.60
TRT II	4989.70	7163.30	77.63	203.98	2.63
TRT III	5200.00	7066.70	66.67	205.18	3.12
TRT IV	5440.90	7283.70	65.81	200.45	3.08
TRT V	5415.00	7875.00	87.86	241.56	2.75
TRT VI	5640.90	7436.40	64.13	222.82	3.47
SEM	131.60	133.525	3.894	5.982	0.135
P values					
Energy	0.043	0.048	0.688	0.162	0.381
Protein	0.175	0.340	0.087	0.422	0.279
Energy × Protein	0.310	0.033	0.156	0.060	0.818

of week six (6) to conduct nutrient digestibility trial. The birds were housed in metabolic cages for 5 days adjustment period before 5 days of excreta collection. Total droppings were collected separately for each replicate daily and pooled together at the end of 5 days of collection for chemical analysis. The proximate composition of the experimental diets was carried out as described by AOAC (2000). Data were subjected to Factorial Analysis of Variance (ANOVA) using the General Linear Model of SAS (2008) package for windows.

Results

The analysed chemical composition of the experimental diets used in 0-4, 4-8, 8-12 and 12-16 weeks are in good agreement with their calculated values (table 3). The performance of poults from 0-4, 4-8, 8-12 and 12-16 weeks is shown in table 4. The performance of poults was not significantly ($p > 0.05$) influenced by energy or protein levels or their combinations. Poults on 2800 kcal ME/kg diet had higher final body weight, daily weight gain and feed intake than poults on 2900 kcal/kg ME irrespective of CP levels and there was no significant interaction between energy × protein levels for any of the performance criteria. Poults on 2800 kcal/kg ME and 30% CP with 93:1 (E/P) ratio had the highest ($p > 0.05$) final body weight (713 g/bird), daily weight gain (23 g/bird) and feed intake (31 g/bird/day), while the least final body weight (573 g/bird) and daily weight gain (18 g/bird/day) was recorded for poults on TRT V with 2900 Kcal/kg ME and 280 g/kg CP (E/P ratio of 104:1). Feed conversion ratio was also not significantly affected by protein, energy or energy/protein ratio.

The performance of poults fed the different energy and protein combinations from 4-8 weeks was not significantly affected by protein levels, but there was a significant effect of energy on the feed intake. There was no significant energy × protein interaction for any of the performance indices. The feed intake of birds fed 3000 kcal/kg ME were higher than birds fed 2900 kcal/kg ME (136 vs. 131 g/bird).

Table 5: Summary of performance of turkeys fed varying energy/protein ratio (0-16 weeks)

Treatments	Initial body weight (g/bird)	Final body weight (g/bird)	Daily weight gain (g/bird)	Daily Feed Intake (g/bird)	Feed conversion ratio g/g
0-16weeks					
TRT I	58.01	7691.7	68.16	159.84	2.35
TRT II	57.95	7163.3	63.44	145.44	2.29
TRT III	55.99	7066.7	62.60	153.04	2.44
TRT IV	56.06	7283.7	64.53	153.19	2.37
TRT V	56.38	7875.0	69.81	167.38	2.40
TRT VI	56.03	7436.4	65.90	159.33	2.42
SEM	0.38	214.77	1.92	3.90	0.04
P values					
Energy	0.15	0.81	0.81	0.58	0.53
Protein	0.41	0.19	0.19	0.22	0.55
Energy × Protein	0.52	0.47	0.47	0.58	0.63

Table 6: Effect of varying energy/protein ratio on apparent digestibility in turkeys (%)

Treatments	Dry Matter	Crude Protein	Crude fiber	Ether extract	Ash
TRT I	90.41	86.45	79.62	88.66	81.17
TRT II	92.5	85.86	78.25	86.35	87.36
TRT III	90.24	76.15	80.19	79.03	81.99
TRT IV	85.24	71.76	77.27	80.72	78.99
TRT V	93.18	89.8	82.48	91.54	68.01
TRT VI	92.7	88.51	88.39	91.11	78.53
SEM	0.972	2.693	1.316	1.769	2.802
P values					
Energy	0.657	0.795	0.132	0.294	0.208
Protein	0.07	0.162	0.114	0.42	0.925
Energy×Protein	0.161	0.101	0.159	0.059	0.464

From 8-12 weeks, there was no significant effect of energy and protein on any of the performance except for final body weight which was significantly affected by energy level and was higher in poults on 3100 kcal/kg ME than those on 3000 kcal/kg ME (5499 vs. 5169 g/bird). There was however no significant energy × protein interaction for any of the performance indices. From 12-16 weeks, there was no significant effect of energy or protein or their ratios on any of the performance indices. There was also no significant energy × protein interaction for any of the performance characteristics. The performance of poults fed diets with varying energy to protein ratios from 0-16 weeks is shown in table 5. Taken together, there was no significant effect of energy, protein or their interaction on any of the performance characteristics of poults from 0-16 weeks of age. There was no significant effect of energy and protein levels or their interaction on any of the performance parameters. The average digestibilities of dry matter, crude protein, crude fibre, ether extract and crude ash were 91, 83, 81, 86 and 79% respectively.

Discussion

The requirement of turkeys for protein decline with age from 28% at hatch to 19% at 12-16 weeks, while that of energy increases from 2800 to 3100 kcal/kg metabolisable energy within the same period (NRC, 1994). However, it is also a well known fact that, poultry species do not attain the high growth rate and laying performance often recorded in the temperate region when raised in the tropical environment even though the genotypes may be the same (Fatufe and Matanmi, 2012). Growth consists mainly of water, protein, fat and mineral accretions. If the growth rate is lower in poults raised under the humid tropics, then it is questionable if the requirements are the same if the levels of intended or realised performance are at variance (Fatufe and Matanmi, 2012). The highest daily feed intake of 31 g/bird and final body weight of 713 g/bird respectively from 0-4 weeks in the present study were lower to the 46 g/bird and 950 g/bird reported by NRC (1994) for unsexed large-type turkeys in the temperate region and this difference could be due to the genotypes of birds used and other environmental factors. Similarly, the daily feed intake and final body weight at 4-8, 8-12 and 12-16 weeks of 133 and 2434 g/bird, 250 and 5334 g/bird, 216 and 7420 g/bird respectively in the present study were lower than 156 and 3500 g/bird, 309 and 7100 g/bird and 452 and 10,750 g/bird respectively of the NRC (1994) for large type turkey of the same age period.

There was no significant difference in the daily weight gain and the feed conversion ratio of the poults fed the different energy to protein ratios in the different phases of growth. This was similar the observation of Peñaranda-Ali *et al.,* (2010). Peñaranda-Ali et al. (2010) fed eight week old male turkeys (Nicholas-700) on two levels of crude protein of 22 and 20% (90 and 100% of NRC recommendations), with energy level of 3000 kcal metabolisable energy/kg diet from 8 – 16 weeks and observed no significant difference in daily weight gain, feed conversion ratio and daily feed intake of birds fed the two crude protein levels. Olomu (1995) reported the energy and protein requirement of 0 - 8 weeks turkeys as 2800 kcal/kg metabolisable energy and 30% crude protein (E/P ratio of 93:1) and 122:1 (2800 kcal metabolisable energy/kg diet and 23% crude protein) for 8 to 16 weeks of age, while Matanmi et al. (2014) reported the energy and protein requirement of 0 - 4 weeks locally adapted poults as 2800 kcal/kg metabolisable energy and 28% protein (E/P ratio of 100:1) in Nigeria. The differences in the energy and protein recommendations could be possibility due to the genotypes of birds used for these studies, their growth rates and other environmental factors. It is also known that different strains of turkey may have different growth pattern due to differences in rate of gain and body composition, which may affect their response to nutrient intake and levels at some point in their growth cycle (Waldroup *et al.,* 1997). Increasing the energy/protein ratio above that of the NRC (1994) did not result in any adverse effect on any of the growth performance characteristics in the present study.

Conclusion

The results of the present study suggested that the energy/protein ratio of exotic turkey reared under the tropical environment may be higher than that of NRC (1994) of 100:1 (2800 kcal/kg ME and 28% crude protein), 116:1 (2900 kcal/kg ME and 26% crude protein), 136:1 (3000 kcal/kg ME and 22% crude protein) and 163:1 (3100 kcal/kg ME and 19% crude protein) for 0-4 weeks, 4-8 weeks, 8-12 weeks and 12-16 weeks respectively and may be as high or more than 107:1 (2800 kcal/kg ME and 26% crude protein), 121:1 (2900 kcal/kg ME and 24% crude protein), 150:1(3000 kcal/kg ME and 20% crude protein) and 177:1(3000 kcal/kg ME and 17% crude protein) for the same age period.

Public brief

The protein and energy contents of poultry diets are an important consideration in feeding birds, because they are the major determinant of the performance characteristics. Protein supplements are however very expensive component of poultry feed and

excessive inclusion of protein supplement in animal feeds will also lead to nitrogen load on the environment as a result of the excretion of excess nitrogen that is not needed for protein accretion/lean meat. There is therefore the need to accurately determine the protein and the energy requirements of turkeys to optimize growth rate at least cost and also to minimize the nitrogen loading of the environment from poultry droppings.

References

A.O.A.C, 2000. Official Methods of Analysis of the Association of official Analytical Chemist Published by the Association of analytical chemists, Washington DC.

Fatufe A. A., Matanmi I. O., 2012. Effect of Varying Crude Protein Levels on the Performance and Carcass Characteristics of Broiler Chicken in the Humid Tropics. Bull. Anim. Hlth. Prod. Afr. 60: 343 – 349

Matanmi I. O., Fatufe A. A., Ogunbiyi T. A., 2014. Effect of dietary energy protein ratio on the performance of Local starting turkeys. Proc. 39th Conf., Nig. Soc. for Anim. Prod. 16 - 19 March, 2014. Babcock Univ. Ilishan-Remo, Ogun State, Nigeria, Pp 622 - 624.

National Research Council, 1994. Nutrient requirements of poultry (9th rev. ed). National Academy Press, Washington DC, USA.

Olomu J. M., 1995. Monogastrics Animal Nutrition – Principles and Practice. JACHEM Publ., Benin city. Pp: 111-148.

Oyedeji, J. O., Atteh J. O., 2005. Response of broilers to feeding manipulations. Int. J. Poult. Sci. 4:91-95.

Peñaranda-Ali F., Santos-Ricalde R., Sarmiento-Franco L., Segura-Correa J., Gutierrez-Triay M., 2010. Effect of Dietary Protein and Lysine on Performance and Carcass Yield of Turkeys. American Journal of Animal and Veterinary Sciences 5: 27-32.

SAS, 2008. SAS Users Guide Statistics, SAS for windows, SAS Inc. Cary, North Califonia, 2008 edition.

Waldroup P. W., Fritts C. A., Kersey J. H., Saleh E. A., Kerr B. J., Kidd M. T., 2003. Evaluation of Crude Protein Needs for Large White Male Turkeys from 16 to 20 Weeks of Age. International Journal of Poultry Science 2: 15-18

Waldroup P.W., England J.A., Waldroup A. L., Anthony N. B., 1997. Response of two strains of large white male turkeys to amino acid levels when diets are changed at three- or four-week intervals. Poultry Science 76:1543–1555

EFFECTS OF DIFFERENT HOUSING SYSTEMS ON GROWTH PERFORMANCE AND CARCASS YIELD OF TWO BREEDS OF TURKEY

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Summary

In the last few decades, a rapid increase in poultry production is experienced due largely to improvements in the management systems. This study thereby investigated the effects of different housing systems on growth performance and carcass yield of exotic and locally-adapted breeds of turkey. A total of 192 unsexed day-old poults (96 each of British United and locally-adapted Turkeys) were purchased and brooded for three weeks. Each breed was then allocated randomly into two treatments of deep litter and wooden cage with 48 poults each which were further subdivided into four replicates of twelve poults each using a 2 x 2 factorial experimental layout in a Completely Randomized Design. The growth performance was significantly ($p<0.05$) influenced by the breeds with exotic breed having higher final weight (6,305.00 g/b), weight gain (60.67 g/b/d) and feed intake (238.57 g/b/d) compared with the final weight (3,541 g/b), weight gain (34.08 g/b/d) and feed intake (138.55 g/b/d) of the locally-adapted turkey breed. Also, significantly ($p<0.05$) higher final weight and weight gain were recorded for the exotic turkey reared on wooden cage at the starter phase. The results on carcass yield showed a significantly ($p<0.05$) higher plucked weight (5,216.67 g/b) in the exotic breed. A better ($p<0.05$) cost-benefit ratio of 3.29 was obtained in the locally-adapted turkey. The study concluded that growth performance indices were better in the exotic than the locally-adapted turkeys. However, with respect to the cost-benefit ratio, the rearing of the locally-adapted turkey in either of the housing system is recommended.

Keywords: locally-adapted turkey, exotic turkey, growth performance, wooden cage, deep litter

EFFETS DES DIFFÉRENTS SYSTÈMES DE LOGEMENT SUR LA PERFORMANCE DE CROISSANCE ET LE RENDEMENT EN VIANDE DE DEUX RACES DE DINDE

Résumé

Les dernières décennies ont vu une augmentation rapide de la production de volailles, due en grande partie aux améliorations des systèmes d'élevage. Cette étude a donc examiné les effets des différents systèmes de logement sur la performance de croissance et le rendement en viande des races exotiques et des races de dinde adaptées aux conditions locales. Au total, 192 dindonneaux d'un jour dont le sexe n'a pas été déterminé (96 pour chacun des groupes de dindes British United et de dindes adaptées aux conditions locales) ont été achetés et couvés pendant trois semaines. Ensuite, les oiseaux de chaque race ont été répartis de manière aléatoire à deux traitements (litière profonde et cage en bois) avec 48 dindonneaux chacun, qui ont été subdivisés en quatre répétitions de douze dindonneaux chacune, en utilisant un dispositif expérimental factoriel 2 x 2 dans un schéma complètement aléatoire. La performance de croissance a été significativement ($p <0,05$) influencée par les races, les races exotiques ayant des valeurs plus élevées de poids final (6,305.00 g / b), de gain pondéral (60,67 g / b / j) et de consommation alimentaire (238,57 g / b / j) par rapport au poids final (3.541 g / b), gain pondéral (34,08 g / b / j) et consommation alimentaire (138.55 g / b / j) des dindes adaptées aux conditions locales. En outre, un poids final et un gain pondéral significativement ($p <0,05$) plus élevés ont été enregistrés pour les dindes exotiques élevées en cage de bois lors de la phase de démarrage. Les résultats concernant le rendement en viande ont montré

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un poids plumé significativement (p <0,05) supérieur (5,216.67 g / b) chez la race exotique. Un meilleur (p <0,05) rapport coûts-bénéfices de 3,29 a été obtenu chez les dindes adaptées aux conditions locales. L'étude a conclu que les indices de performance de croissance étaient meilleurs chez les dindes exotiques par rapport aux dindes adaptées aux conditions locales. Cependant, en ce qui concerne le rapport coûts-bénéfices, l'élevage de dindes adaptées aux conditions locales dans l'un ou l'autre système de logement est recommandé.

Mots-clés : dinde adaptée aux conditions locales, dinde exotique, performance de croissance, cage en bois, litière profonde

Introduction

Indigenous chickens have remained predominant in the developing world (Do, 2005; Bett *et al.,* 2012) despite the introduction of the exotic strain. Local chickens have a slow growth rate thereby attaining a market body weight at a much later age when compared with the exotic meat strain. The slow growth has been shown to result into tastier and tougher meat (Wattanachant *et al.,* 2004; Saowakon *et al.,* 2008); attributes which are concomitant with preferences of many chicken consumers. UBOS (2009) reported an increase in the number of producers and consumers interest in local chicken products whereas Mugga (2007) and Kyarisiima et al. (2004; 2011) reported a big market niche for local chicken products because their meat is perceived to be tastier than that from exotic strain.

In Nigeria, there is no known discriminatory legislation against the production of turkey and consumption of its meat but they are very scarce to find. It was thereby opined that the potentials of locally-adapted turkeys cannot be overlooked considering the huge foreign exchange implication of the imported exotic stocks. However, scarcity of the local strain of turkey could be due partly to the fact that chickens are grown so well that there seems to be no reason to consider any other poultry species, and partly because most exotic turkeys have been so highly bred for intensive production that the resulting birds are inappropriate for extensive production (Peters *et al.,* 2002). These exotic turkeys are characterized by early maturity, high productivity but low tolerance to change in environmental condition; disease, pest and parasites. The problems associated with reliance on exotic breeds for turkey industry in Nigeria include poor performance, importation of disease if birds are not well quarantined and the need to replenish stock by importation which in turn affects the country's foreign exchange earnings.

Alternative poultry production involves a cage-free environment or other access to the outdoors as alternative to conventional poultry housing and cages. It is an important aspect of sustainable agricultural production that enhances farm income while protecting the environment and addressing consumers' concerns. It could be practiced on a larger scale but it is often on a small-scale and sometimes integrated into a diversified farm. In a recent study (Sogunle *et al.,* 2013) on cockerel chickens, it was revealed that free range could be an alternative to confinement housing which was found environmentally sound, economically viable and focused on low-input strategies.

It is noteworthy that housing system is dependent on the market for different categories of birds and the practical experiences with competing model (Tauson, 2005). Therefore, for any housing or production system to be considered good, birds must be free from stress and disease. Birds need to be able to grow, sleep and lay eggs in comfort. Pasture rearing is thereby a cornerstone to this approach. This study thereby seeks to evolve an acceptable recommendation from the comparison of the growth performance and carcass yield of raising exotic and locally-adapted breeds of turkey in different housing systems.

Materials and methods

Experimental Site

The experiment was carried out at the

Turkey Unit of the Directorate of University Farm (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of Southern - Western on Latitude 70 10' N and Longitude 30 2' E and altitude of 76m above the sea level (Google Earth 6.0, 2014). The climate is humid with a mean annual rainfall of about 1037 mm and mean temperature and humidity of 34.7 0C and 83%, respectively.

Experimental birds and management

A total of 192 unsexed day old poults (96 each of locally-adapted and British Unit Turkeys) were purchased from Agrited hatchery Nigeria Limited in South-western Nigeria. The poults were intensively brooded together for three weeks on floor with space requirements of 0.25 square metre per poult. At the end of the third week each breed of poults were on weight equalization basis (balancing for the sexes) and allocated randomly to two treatment groups of deep¬-litter and wooden cage housing systems of 48 poults each. Each treatment group was further divided into four replicates of twelve (12) poults each. Routine and occasional management practices in turkey production were carried out as at when due. Feeders and drinkers were cleaned daily and fresh feed and water were supplied daily. Litter materials (wood shavings) were changed forthnightly and as at when due throughout the experimental period. The birds were fed commercially prepared turkey diets containing 26.6% CP and 12.1 MJ/kg ME at weeks 3 to 8 (starter phase), and 19.15% CP and 12.54 MJ/kg ME at weeks 9 to 16 (grower phase).

Dimensions of the housing systems

Each wooden cage as well as each deep litter pen has a dimension of 1m x9m which is 9m2 that housed 12 turkeys. The height of the wooden cage which was made of wire mesh and wood with galvanized metal at the base was 2.5m from the floor to the roof.

Experimental Design

The experimental layout was a 2x2 factorial arrangement that contained two housing systems (wooden cage and deep litter) and two breeds of turkeys (locally-adapted and exotic).

Data Collection

The following measurements were taken on the growth performance of the poults at starter and grower phase:

Determination of Growth Performance

i. Weight gain (g)

The initial body weight of each of the group of turkeys was taken while subsequent body weights were recorded on weekly basis.

Weight gain = Final weight - Initial weight.

ii. Feed Intake (g)

Feeds were given to each of the group of poults while left over of feeds were weighted to determine daily feed intake and consequently weekly feed intake. This was calculated using the formula:

$$\text{Feed intake per bird} = \frac{\text{Feed supplied - Left over of feed}}{\text{Number of birds}}$$

iii. Feed conversion ratio (FCR) determination

The FCR of each group of poults was determined by calculating the ratio of feed intake to weight gain and thus calculated as:

iv. $$\text{Feed conversion ratio (FCR)} = \frac{\text{Total Feed intake (g)}}{\text{Total body weight gain (g)}}$$

$$\text{Percentage mortality} = \frac{\text{Number of dead birds per replicate X 100}}{\text{Initial number of bird per replicate}}$$

Cost-Benefits ration

The cost analysis of each breed of turkeys was estimated using the prevailing market prices at the time of the study. Brooding cost and the cost of feed were recorded, and the feed intake of each turkey in the course of the experiment was used to multiply the cost per kg of feed to obtain the cost of feed

consumed per turkey. The production cost per kg live weight gain was calculated as an estimate of cost of day old poults, brooding cost, drugs, vaccines and feeds. A dollar was equivalent to N170 at the time of the study

Statistical Analysis

Data collected were subjected to Completely Randomized Design. Significantly ($P<0.05$) different means were separated using Duncan's Multiple Range Test as contained in SAS (2003) package. Data collected on the cost benefit ratio were subjected to studentized t-test at 5% level of significance while changes in weight of the breeds of turkey in the different housing systems were descriptively expressed using line charts.

$$Y_{ijk} = \mu + Ai + Bj + (AB)_{ij} + E_{ijk}$$

where:

Y_{ijk} = observed value of a dependent variable

μ = Population mean

A_i = Effect of the ith housing systems group (i=1,2)

B_j = Effect of the jth breed (j = 1,2)

$(AB)_{ij}$ = Effect of the interaction between housing systems and breeds

E_{ijk} = Random error associated with each observation.

Results

Growth performance of locally-adapted and exotic turkey breeds on deep litter and wooden cage at the starter phase

The main effects of breed and housing system on the growth performance of turkey at the starter phase (3-8 weeks) are shown in Table 1. The breeds were significantly ($p<0.05$) different in all the parameters considered including the initial weight and the body temperature which were higher in the exotic turkey from day-old. Although, exotic turkey had a better ($p<0.05$) feed conversion ratio than locally-adapted turkey but it recorded more mortality (20.33%) than the locally-adapted turkey (11.88%). In the housing system, significantly ($p<0.05$) higher final weight (g/b) and weight gain (g/b/d) were obtained in turkeys reared on wooden cage.

In the interaction effects between breed and housing system on the growth performance of turkey at starter phase (Table 2), significantly ($p<0.05$) highest final weight (g/b), weight gain (g/b/d) and feed intake (g/b/d) including mortality (%) were observed in exotic turkey on wooden cage. Locally-adapted turkeys on deep litter recorded lowest values in final weight (g/b), weight gain (g/b/d), and feed intake (g/b/d). The lowest ($p<0.05$) mortality of 9.10 % was recorded in locally-adapted turkeys on wooden cage.

Growth performance of locally-adapted and exotic turkey breeds on deep litter and wooden cage at the grower phase (9-16 weeks)

In Table 3, the main effects of breed and housing system on the growth performance of turkey at the growing phase are shown. Exotic breed had significantly ($p<0.05$) higher initial weight (g/b), final weight (g/b), weight gain (g/b/d) and feed intake (g/b/d). On the other hands, significantly ($p<0.05$) higher initial weight (2026.69 g/b) was recorded in turkeys reared on wooden cage.

In the effects of interaction between breed and housing system on growth performance of turkey at growing phase (Table 4), significant differences were obtained in the initial weight, final weight, weight gain, feed intake and body temperature (0C). The highest ($p<0.05$) weight gain of 61.45g/b was obtained in exotic breed reared on deep litter while the lowest value of 32.11g/b/d was obtained in locally-adapted turkey reared on wooden cage.

Figure 1 depicts exotic turkey having no marked differences in weight changes in the housing systems for the period of the experiment. This same trend is observed in Figure 2 for locally-adapted turkey in the housing systems at the 16th week. However, from the 6th to the 14th week, locally-adapted turkey reared in wooden cage had relatively higher weight changes than those reared on

Table 1: Main effects of breed and housing system on the growth performance of turkey at the starter phase (3-8weeks)

	Breed		Housing System	
Parameter	**Locally-adapted**	**Exotic**	**Deep litter**	**Wooden cage**
Initial Weight (g/b)	208.60±2.39^{b}	289.05±14.92^{a}	255.12±0.89	242.53±19.47
Final Weight (g/b)	1394.07±62.00^{b}	2482.91±61.11^{a}	1850.28±244.4^{b}	2026.69±251.49^{a}
Weight gain (g/b/d)	21.16±1.07^{b}	39.17±1.15^{a}	28.48±3.99^{b}	31.86±4.21^{a}
Feed intake (g/b/d)	70.44±2.25^{b}	94.17±5.08^{a}	78.67±4.69	85.93±7.73
Feed conversion ratio	3.35±0.15^{a}	2.40±0.10^{b}	2.95±0.30	2.80±0.18
Mortality (%)	11.88±5.54^{b}	20.33±9.11^{a}	13.39±5.08	18.81±9.6
Body temperature (^{0}C)	37.50±00^{b}	41.50±0.00^{a}	39.50±0.00	39.50±0.89

^{a,b} Means in the same row with different superscripts differ significantly (P<0.05)

Table 2: Effects of interaction between breed and housing system on the growth performance of turkey (3-8weeks)

	Exotic		Locally-adapted	
Parameter	**Deep litter**	**Wooden Cage**	**Deep litter**	**Wooden cage**
Initial Weight (g/b)	303.15±9.78^{a}	274.96±28.62^{a}	207.10±1.05^{b}	210.10±5.02^{b}
Final Weight (g/b)	2389.44±78.18^{a}	2576.37±61.90^{a}	1311.11±45.74^{b}	1477.02±101.37^{b}
Weight gain (g/b/d)	37.25±1.50^{a}	42.01±0.83^{a}	19.71±0.79^{b}	22.62±1.72^{b}
Feed intake (g/b/d)	86.71±5.25^{ab}	101.62±6.78^{a}	70.64±4.20^{b}	70.24±2.72^{b}
Feed conversion ratio	2.32±0.07^{b}	2.48±0.21^{b}	3.58±0.22^{a}	3.12±0.13^{a}
Mortality (%)	12.13±8.02^{b}	28.53±16.84^{a}	14.66±7.94^{b}	9.10±9.10^{c}
Body temperature (^{0}C)	41.50±0.00^{a}	41.50±0.00^{a}	37.50±0.00^{b}	37.50±0.00^{b}

^{a,b} Means in the same row with different superscripts differ significantly (P<0.05)

Table 3: Main effect of breed and housing system on the growth performance of turkey at the growing phase (9-16weeks)

	Breed		Housing System	
Parameter	**Locally-adapted**	**Exotic**	**Deep litter**	**Wooden cage**
Initial Weight (g/b)	1394.07±62.00^{b}	2482.91±61.11^{a}	1850.28±244.49^{b}	2026.69±251.49^{a}
Final Weight (g/b)	3541.00±68.28^{b}	6305±291.65^{a}	4922.17±656.79	4925.00±649.78
Weight gain (g/b/d)	34.08±1.72^{b}	60.67±4.14^{a}	48.76±6.89	46.00±6.50
Feed intake (g/b/d)	138.55±4.31^{b}	238.57±14.27^{a}	181.71±24.35	195.40±24.70
Feed conversion ratio	4.14±0.32	3.94±0.10	3.76±0.20	4.32±0.22
Mortality (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Body temperature (^{0}C)	40.00±00	43.00±0.00	41.50±0.00	41.50±0.07

^{a,b} Means in the same row with different superscripts differ significantly (P<0.05)

Table 4: Effects of interaction between breed and housing system on growth performance (9-16 weeks)

	Exotic		Locally-adapted	
Parameter	**Deep litter**	**Wooden Cage**	**Deep litter**	**Wooden cage**
Initial Weight (g/b)	2389.44±78.18[a]	2576.37±61.90[a]	1311.11±45.74[b]	1477.02±101..37[b]
Final Weight (g/b)	6261.00±589.49[a]	6350.00±275.38[a]	3583.33±130.17[b]	3500.00±68.06[b]
Weight gain (g/b/d)	61.45±8.33[a]	59.90±3.97[a]	36.06±2.66[b]	32.11±1.97[b]
Feed intake (g/b/d)	230.01±24.60[a]	247.12±18.43[a]	133.42±5.28[b]	143.69±6.22[b]
Feed conversion ratio	3.77±0.13	4.12±0.07	3.76±0.0.44	4.52±0.44
Mortality (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Body temperature (^{0}C)	43.00±0.00	43.00±0.00	40.00±0.00	40.00±0.00

[a,b] *Means in the same row with different superscripts differ significantly (P<0.05)*

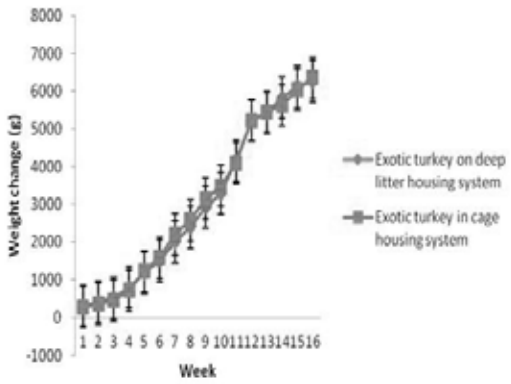

Figure 1: Effects of housing system on weekly weight change of exotic turkey

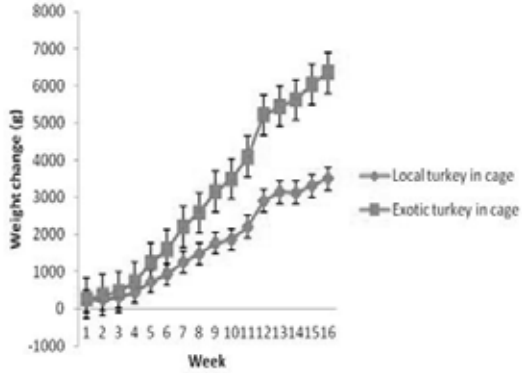

Figure 3: Effects of deep litter housing system on weekly weight change of local and exotic turkey.

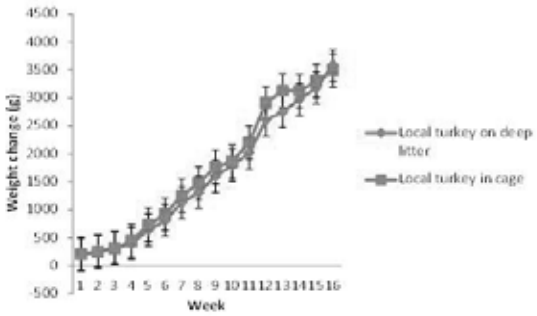

Figure 2: Effects of housing systems on weekly weight change of locally-adapted breed of turkey

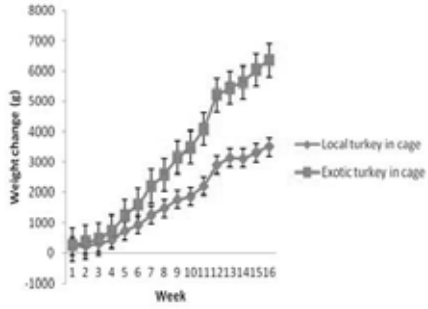

Figure 4: Effects of cage housing system on weekly weight change of local and exotic turkey

deep litter. In Figure 3, marked difference could be observed in the weight changes between locally-adapted turkey and exotic turkey reared on deep litter housing system and this same trend is depicted by the two breeds of turkey reared on wooden cage as shown in Figure 4.

Carcass yield of locally-adapted and exotic turkey breeds on deep litter and wooden cage

Table 5 shows the main effects of breed and housing system on carcass yield of turkey. In the breed, higher ($p<0.05$) significant effects were observed in live weight (5,916.67g), and plucked weight (5216.67g) in the exotic breed than the locally-adapted turkeys but the dressing percentage was not significantly ($p>0.05$) different. In the cut-up parts, higher significant ($p<0.05$) differences were obtained in the breast meat (17.90%) and shanks (3.40%) in the exotic breed of turkey than the locally-adopted turkey. However, higher significant ($p<0.05$) value was obtained in the head (2.78%) of the locally-adopted turkey than the exotic breed (1.72%). Also, in the organs, higher ($p<0.05$) significant value was obtained in the proventriculus (0.23%) of exotic breed than the locally-adopted turkey. The housing system did not significantly ($P>0.05$) influence all the parameters considered.

In the effects of interaction between breed and housing systems on the carcass yield of turkey (Table 6), significant ($p<0.05$) differences were obtained in live weight, plucked weight, head, neck, drumsticks, thighs, shanks, proventriculus and the small intestine across the treatments. Exotic turkey on wooden cage had the highest ($p<0.05$) values in live weight, plucked weight, drumsticks and thighs. The dressing percentage, wings, back, breast, liver, heart, gizzard, kidney, lungs, spleen, caecum and large intestine were not significantly ($p>0.05$) different across the treatments.

Cost-benefit ratio of rearing locally-adapted and exotic turkey breeds

In Table 7, the cost-benefits ratio of rearing locally-adapted and exotic turkeys is shown. Significantly ($p<0.05$) higher total feed intake/bird (g), cost of feed consumed (N), initial cost, total cost/bird (N), average final

Table 5: Main effects of breed and housing systems on carcass characteristics of turkey

	Breeds		Housing Systems	
Parameter	**Locally-adapted**	**Exotic**	**Deep litter**	**Cage**
Live weight (g/b/)	3600±0.00[b]	5916.67±0.00[a]	4750.00±560.80	4766.67±603.13
Plucked weight (g/b)	3200±106.45[b]	5216.67±435.44[a]	4150.00±515.59	4266.67±583.47
Dressing percentage (%)	82.37±1.80	80.68±2.56	80.69±2.48	82.36±1.90
Cut-up parts[1]				
Head	2.78±0.05[a]	1.72±0.10[b]	2.23±0.22	2.27±0.27
Neck	9.27±0.62	10.14±0.63	9.11±0.55	10.30±0.46
Wings	9.09±0.51	10.17±0.37	9.41±0.53	9.85±0.46
Drumsticks	9.15±0.15	11.22±0.50	9.61±0.52	10.76±0.69
Thighs	8.43±0.41	9.44±0.63	8.37±0.30	9.50±0.55
Breast	13.91±0.73[b]	17.90±1.52[a]	15.00±1.63	16.81±1.20
Shanks	2.78±0.05[b]	3.40±0.21[a]	4.69±0.30	5.32±0.36
Organs[2]				
Liver	1.16±0.10	1.03±0.85	1.17±0.11	1.02±0.69
Heart	0.35±0.02	0.32±0.02	0.30±0.01	0.37±0.01
Gizzard	2.29±0.19	1.92±0.10	2.16±0.19	2.05±0.01
Kidney	0.36±0.01	0.33±0.02	0.34±0.02	0.35±0.01
Lungs	0.50±0.06	0.44±0.05	0.55±0.05	0.42±0.04

	Breeds		Housing Systems	
Parameter	**Locally-adapted**	**Exotic**	**Deep litter**	**Cage**
Spleen	0.16±0.03	0.11±0.03	0.13±0.04	0.13±0.03
Ceacum	0.17±0.02	0.17±0.03	0.15±0.02	0.19±0.03
Proventriculus	0.03±0.01b	0.23±0.02a	0.27±0.01	0.26±0.02
Small intestine	1.98±0.09	1.72±0.11	1.97±0.11	1.73±0.10
Large intestine	0.71±0.03	0.50±0.03	0.66±0.03	0.64±0.04

[a,b] Means in the same row with different superscripts differ significantly (P<0.05)
[1,2]: Percentage of the live weight

Table 6: Effect of interaction between breed and housing systems on carcass characteristics of turkey

	Exotic		Locally-adapted	
Parameter	**Deep litter**	**Cage**	**Deep litter**	**Cage**
Live weight (g/b)	5766.67±731.05[a]	6066.67±352.76[a]	3733.33±66.66[b]	3466.67±64.66[b]
Plucked weight (g/b)	4900.00±873.68[ab]	5533.33±290.59[a]	3400.99±57.73[bc]	3000.88±55.47[c]
Dressing percentage	77.47±4.52	83.90±1.40	83.91±0.29	80.82±03.72
Cut-up parts[1]				
Head	1.78±0.20[b]	1.65±0.10[b]	2.68±0.04[a]	2.88±0.05[a]
Neck	4.03±0.13[b]	4.86±0.68[ab]	5.36±0.04a	5.77±0.05[a]
Wings	9.85±0.29	10.49±0.70	8.96±1.07	9.21±0.39
Back	10'96±0.70	9.31±0.90	9.64±1,07	8.91±0.81
Drumstick	10.23±0.48[ab]	12.12±0.48[a]	8.91±0.81[b]	9.40±0.57[b]
Thigh	8.53±0.63[ab]	10.35±0.89[a]	8.21±0.16[b]	8.66±0.16[ab]
Breast	16.60±2.90	19.20±1.20	13.40±1.53	14.43±0.27
Shanks	3.57±0.41[a]	3.31±0.20[ab]	2.68±0.04[b]	2.88±0.05[ab]
Organs[2]				
Liver	1.09±0.16	0.97±0.07	1.24±0.18	1.07±0.12
Heart	0.29±0.03	0.36±0.01	0.32±0.02	0.38±0.03
Gizzard	1.97±0.14	1.88±0.18	2.36±0.36	2.21±0.20
Kidney	0.33±0.04	0.34±0.01	0.36±0.03	0.37±0.01
Lungs	0.46±0.08	0.43±0.07	0.63±0.02	0.41±0.08
Spleen	0.13±0.07	0.09±0.03	0.14±0.04	0.17±0.04
Ceacum	0.15±0.04	0.18±0.06	0.14±0.02	0.20±0.04
Proventriculus	0.24±0.03[ab]	0.21±0.03[b]	0.29±0.01[ab]	0.31±0.02[a]
Small intestine	1.80±0.20[ab]	1.63±0.14[b]	2.14±0.03[a]	1.83±0.14[ab]
Large intestine	0.60±0.03	0.58±0.06	0.71±0.04	0.70±0.06

[a,b] Means in the same row with different superscripts differ significantly (P<0.05)
[1,2]: Percentage of the live weight.

weight/bird (kg) and income/bird (N) were obtained in exotic turkey. In addition, a higher but poorer ($p<0.05$) cost-benefits ratio (3.91) was obtained in exotic turkey than in the locally-adapted turkey.

Discussion

From the results of this study, the difference in higher weight gain observed in exotic breed than the locally-adapted breed is certainly breed-specific and in the growth associated with the increase in feed intake. This could also be corroborated by the findings of Saowakon et al. (2008) that locally-adapted birds have a slow growth rate when compared with the exotic meat strain thus attaining a marketable body weight at much later age. The final body weight recorded for locally-adapted breed is in line with the findings of Karki (2005) who reported that at 16th week of age locally-adapted turkey will have highest body weight after which it deteriorates progressively. In addition, the growth performance exhibited by the exotic breed of turkey in this study resulted from early maturity and high productivity. Body weight in the exotic turkey was significantly higher than that of locally-adapted breed in this study and it is supported by the fact that locally-adapted poultry have gone through more of natural selection for survival to the tropical climate rather than artificial selection for productivity (Ibe, 1990). Mortality rate was higher in the exotic turkey on wooden cage housing system which was in contrast with the findings of Garber et al. (2003) that birds reared under deep litter floor systems had higher risk of infections compared to those kept in cage system. However, Namata et al. (2008) reported higher risk of contamination under cage system. The non-significant effects in the growth performance of the breeds in the two housing systems as shown in the interactive effects supported the reports by Fanatico et al. (2005) that showed no differences in the final weight of indoor and outdoor birds. The growth of the exotic breed of turkey was influenced mainly by breed effect and not by housing systems. Exotic and locally-adapted breed of turkeys reared in wooden cage housing systems performed better than those reared on deep litter. This study suggest that the effect of choice of housing systems on feed consumption is of importance as more feed was consumed in wooden cage than deep litter for the exotic breed while consumption was high on deep litter for the locally-adapted breed. The body temperature of the birds in this study fell within the range of 41-42oC for optimal performance of birds. The difference in the temperature of the turkeys in both breeds might have been due to adaptation, it could also be as a result of the thermal stress (high internal/external temperature).

The Exotic breed had higher live weight, plucked weight, proventriculus, shanks and drumstick. The possible explanation for the differences between locally-adapted and exotic breed is the body size. In this study, the cage reared turkey had heavier body weight, lower feed conversion and higher meat yield than those reared on deep litter. This result is in contrast with the report by Sogunle et al. (2008) where broilers reared on deep litter showed better feed conversion ratio and lower mortality compared to those reared in cage. In addition, Santos et al. (2008) also corroborated it in his report that litter reared birds not only have significant heavier gizzard and proventriculus than cage reared birds, but also have improved feed conversion ratio and heavier breast muscle relative to body weight. The exotic breeds had higher value of live weight, thighs and breast meat percentage.

In this study, feed alone accounts for between (60-70%) of the production cost. The rearing of the locally-adapted turkey was found profitable in the long run with a better cost-benefits ratio. The high cost of production of the exotic turkey results narrow profit margin. This could in the long run lead to a collapse of the once prosperous poultry industry with high cost of production occasioned by higher feed intake. Of course, high cost of feed in poultry industry in Nigeria is a major challenge facing the poultry farmer. Hence, frequent and sometimes unwarranted increases in the prices of ingredients have contributed substantially to the difficulties experienced by poultry farmers in Nigeria.

Conclusion

1. There were breed differences in the growth performance indices with better growth performance in the exotic turkey than the locally-adapted turkey. In addition, exotic turkey on wooden cage performed better than exotic turkey on deep litter and locally-adapted turkey on both housing systems at the starter phase.
2. Exotic turkey yielded more in term of the carcass component than the locally-adapted turkey but recorded similar dressing percentage with the locally-adapted turkey.
3. The locally-adapted turkey recorded better cost-benefits ratio than the exotic turkey.

Recommendations

The followings are recommended based on the result of the study:

1. The two housing systems of deep litter and wooden cage are recommended for turkey production. However, for a better performance and higher meat yield, the exotic turkey could be reared on wooden cage.
2. In terms of the cost-benefits ratio and reduced mortality, the rearing of the locally-adapted turkey in any of the housing systems is recommended.

Impact

The study further explored an alternative to turkey production in the use of wooden cage which compared favourably with the rearing of turkey on deep litter housing system. It has also shown that rearing of locally-adapted turkey would be a better option to the exotic breeds of turkey for the rural resource-poor poultry farmer thereby encouraging its profitable production.

References

Bett, H. K., Bett, R. C., Peters, K. J., Kahi, A. K. and Bokelmann, W. 2012. Linking utilization and conservation of Indigenous chicken resources to vale chains. Journal of Animal Production Advances, 2: 33-52.

Do, V.M. 2005. Effects of supplementation, breed, season and location on feed and performance of scavenging Local Chickens in Vietnam. A Doctorial Thesis submitted to Swedish University of Agricultural Sciences, Uppsala, Sweden. hhpt:dissepsilon.slu.searcive/00000953/01/ MinhGeneraldiscussion 2005. pdf.

Fanatico, A.C., Pillai, P.B., Cavitt, L.C., Owens, C.M and Emmert, J. L. 2005. Evaluation of slower-growing broiler genotypes grown with and without outdoor access: growth performance and carcass yield. Poultry Science, 84:1321-1327.

Garber, L., Smelzer, M., Fedorka-Cray, P., Ladely, S. and Ferris, K. 2003. Salmonella enteric serotype Enteritidis in table egg layer house environments and in mice in US layer houses and associated risk factors. Avian Diseases 47, 134 -142.

Google Earth, 2014. http://www.google.earth

Ibe, S. N. 1990. Utilizing local poultry genetic resources in Nigeria. In: Proceedings of 4th World Congress on genetics applied to livestock production. Edinburgh, Scotland . 108- 112.

Karki, M. 2005. Growth, efficiency of feed utilization and economics of different periods of turkeys. Nepal Agricultural Research Journal (SAS/N), 6:84-88.

Kyarisiima, C. C., Kugonza, D. R., Twesigye, C. K. 2004. The potential role of the Ugandan Indigenous Chicken in Poverty Alleviation. Uganda Journal, 50: 85-90.

Kyarisiima, C. C., Nagujja, F. A., Magala, H., Kwizera, H., Kugonza, D. R. and Bonabana-Wabbi, J. 2011. Perceived tastes and preferences of chicken meat in Uganda. LRRD, 23 (11) 2011, www.lrrd.org/ lrrd23/11/kyar23242.htm

Mugga, R. 2007. Uganda sees a Market for Indigenous Birds. World Poultry 23:1 http//www.world poultry. net.

Namata, H., Meroc, E., Aerts, M., Faes, C., Abrahantes, J. C., Imberechts, H. and Mintiens, K. 2008. Salmonella in Belgian laying hens: An identification of risk factors. Preventive Veterinary Medicine. 83:323–336.

Peters, S. O. Ikeobi, C. O. N and Bamkole, O. O. 2002. Smallholder local turkey production in Ogun

State. In: Issues in family poultry Research and Development. Proceedings of the international network for family poultry development at Senegal, Dec. 9-13, 1997, pp 173-183.

Santos, A. L., Sakomura, N. K., Freittas, E. R., Fortes, C. M. S. and Carrilho, E.N.V.M. 2005. Comparison of free range broiler chicken strains raised in confined or semi-confined systems. Brazilian Journal of Poultry Science, 7:85-92.

Saowakon, W. 2008. Factors affecting the quality characteristics of Thai Indigenous chickens. Suranaree Journal of Science and Technology, 15 : 317-322.

SAS, 2003. SAS® User's Guide: Statistics Version 9.1. SAS Institute Cary, NC, USA.

Sogunle, O.M., Egbeyale, L.T., Bajomo, T.T., Bamigboje, O.V. and Fanimo, A.O. 2008. Comparison of the performance, carcass characteristics and haematological parameters of broiler chicks reared in cage and floor. Pakistan Journal of Biological Science, 11:480-483.

Sogunle, O. M., Olaniyi, O. A., Egbeyale, L. T., Akinola, O. S., Shittu, T. A., Abiola, S. S., Ladokun, A. O. and Sobayo, R. A. 2013. Free range and deep litter production systems: Effect on performance, carcass yield and meat composition of cockerel chickens. Tropical Animal Health and Production, 45 (1) 281 -288.

Tauson, R. 2005. Management and housing systems for layers – effects on welfare and production. World's Poultry Science Journal, 61: 477 – 490.

UBOS (Uganda Bureau of Statistics) 2009. Uganda National Household Survey 2008 Report on Agricultural Module.

Wattanachant, S. Benjakul, S. and Ledward, D. A. 2004. Composition, Colour and Texture of Thai Indigenous and Broiler Chicken Muscles. Poultry Science, 83:123-128

GROWTH RATE AND MANURE QUALITY OF SMALL RUMINANTS UNDER RURAL PRODUCTION IN THE GAMBIA

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Abstract

A three-month long on-farm participatory study was undertaken in four villages within The Gambia. The objectives of the study were to determine the average daily weight gain (ADWG) and faecal outputs of small ruminants under the traditional management system.

Thirty four young bucks and rams used in this study are 6 to 12 months old were owned and managed by 18 farmers. They comprise of Djallonke sheep (DS), Crossbred sheep (Djallonke x Sahelian sheep) (DSS), and West African Dwarf Goats (WADG). All the animals were weighed every fortnight. Their faecal droppings were collected either overnight or over 24 hour period, three times a week. The mineral contents of the collected faeces were determined. Collected data was partitioned on the basis of species (sheep vs goats) and sheep breeds, and analyzed using descriptive statistics and linear regression models.

Results showed no significant differences ($p > 0.05$) in ADWG: 25.6g for DS, 40.0g for DSS, and 34.8g for WADG goat. Overnight faecal outputs were: 218g for DS, 151g for DSS and 129g for WADG. The 24 hour faecal outputs were 487g, 347g and 275g for the DSS, DS and the WADG, respectively.

Goat manure contained 2.3% nitrogen, 1.0% phosphorus and 0.9% potassium; while sheep manure had 2.0% nitrogen, 0.9% phosphorus, and 0.6% potassium. Nitrogen and potassium contents of sheep manure were significantly ($p < 0.05$) lower than goats manure. The mineral contents of Djallonke and Crossbred rams manure were not significantly different ($p > 0.05$). In conclusion, the crossbreed sheep grew faster and produced more daily faecal output than both WADG and Djallonke sheep. Follow up sheep and goat fattening trials utilising leguminous dual purpose varieties of cowpea and groundnut are recommended. In addition, participatory studies like this should have a control component on station for comparison purposes.

Keywords: Average Daily Weight Gain, Faeces, Gambia, Minerals, Goat and Sheep

TAUX DE CROISSANCE ET QUALITÉ DU FUMIER DES PETITS RUMINANTS EN SYSTÈME DE PRODUCTION RURALE EN GAMBIE

Résumé

Une étude participative de trois mois a été réalisée dans quatre villages de la Gambie. Les objectifs de l'étude étaient de déterminer le gain pondéral moyen quotidien (GMQ) et les quantités d'excréments des petits ruminants élevés en système traditionnel.

Trente-quatre jeunes béliers et boucs utilisés dans cette étude étaient âgés de 6 à 12 mois et étaient détenus et gérés par 18 éleveurs. Ils comprenaient des moutons Djallonké (DS), des moutons croisés (Djallonke x moutons sahéliens : DSS), et des chèvres naines d'Afrique de l'Ouest (WADG). Tous les animaux étaient pesés tous les quinze jours. Leurs excréments étaient recueillis soit la nuit soit sur une période de 24 heures, trois fois par semaine. Les teneurs en minéraux des excréments recueillis ont été déterminées. Les données recueillies ont été réparties suivant les espèces (moutons vs chèvres) et les races ovines, et analysées à l'aide de statistiques descriptives et de modèles de régression linéaire.

Les résultats n'ont montré aucune différence significative ($p > 0,05$) dans les WADG : 25,6 g pour les DS, 40,0 g pour les DSS, et 34.8g pour les chèvres WADG. Les quantités d'excréments produits la nuit étaient : 218g pour DS ; 151g pour DSS ; et 129g pour WADG. Les quantités des excréments produits sur 24 heures étaient 487 g ; 347g ; et 275g, respectivement pour DSS, DS et WADG.

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Les excréments des chèvres contenaient 2,3% d'azote, 1,0% de phosphore et 0,9% de potassium ; tandis que ceux des moutons contenaient 2,0% d'azote, 0,9% de phosphore et 0,6% de potassium. Les teneurs en azote et en potassium des excréments de moutons étaient significativement ($p < 0,05$) inférieures à celles des excréments des chèvres. Les teneurs en minéraux des excréments des béliers Djallonké et croisés n'étaient pas significativement différentes ($p > 0,05$). En conclusion, les moutons croisés avaient une croissance plus rapide et produisaient plus d'excréments par jour que les WADG et les moutons Djallonké. Des essais de suivi sur l'engraissement des moutons et des chèvres utilisant des variétés de légumineuses et arachides à fonction double sont recommandés. En outre, des études participatives comme celle-ci devraient avoir une composante de groupes témoins à des fins de comparaison.

Mots-clés : gain pondéral moyen quotidien, excréments, Gambie, minéraux, chèvre et mouton

Introduction

Small ruminant production plays a major socio-economic role in the promotion of improved livelihood and welfare for many actors involved in the sheep and goats value chains. Small ruminants are usually the first line of defence in providing cash for many households to purchase food during crop failures. They serve as a safety net in terms of food security and as asset for several socio-economic engagements. Whilst male farmers are more involved in cattle production, women and children dominate the management of small ruminants. According to Jaitner et al. (2001), women own 52% of sheep, 67% of goats, and 43% of both sheep and goats in The Gambia.

Health problems particularly Peste des Petits Ruminants (PPR) and helminthoses during the rainy season, and poor management with regards to grazing and housing constitute formidable constraints to production and productivity . Poor grazing management and lower weight gains in small ruminants have been described as deterrents to increase productivity during rainy season in The Gambia Osaer et al. (1999). Small ruminants are usually collectively herded or individually tethered during the rainy season, but are more frequently managed on free range system during the dry season.

This study was undertaken as a scoping study to determine the average daily weight gain and faecal outputs of sheep and goats under farmers' traditional management system.

Materials and Methods

Study sites

The three-month long (September to November 2012) on-farm study was undertaken in four villages within two districts of The Gambia. The four villages are Bassik and Dibba Kunda Fulla in Sabakh Sanjal District (North Bank Region), Chamen and Palelei in Nianija District (Central River Region North).

Bassik is predominantly inhabited by Mandinkas and Wollofs, with a population of about 1000 people, practicing mixed crop and livestock production. Dibba Kunda Fulla is a community of Fulani people with also about 1000 inhabitants that also cultivate crops and rear animals. There is also a primary health post and flow of pipe borne water supply. Chamen is the biggest village out of the four, houses the chieftaincy of Nianija district, with portable water supply, major health centre, lower basic school cycle, agriculture and livestock extension agents. It is also inhabited by Fulani people with a population of about 2000 people that also practice mixed crop cultivation and livestock production. Palelei is a small village of about 1000 people comprising Fulani people that are also engaged in crop cultivation and livestock management. The cereals cultivated in these villages are maize, millet and rice; legumes are groundnut hay and cowpea; and the ruminant species are cattle, goats and sheep.

Study animals

A total of 34 young bucks and rams were monitored during this study. They comprised of 11 West African Dwarf Goat (WADG) bucks, 13 Djallonke sheep (DS) rams, and 10 Crossbred rams (DSS) (Djallonke sheep x Sahelian) as in table 1.

Most of the monitored animals were collectively grazing in the communal pastures through group herding and the rest tethered by

rope on fixed pegs. Grazing or tethering lasted for five hours daily from mid-day to 5 p.m. within the fallow lands near the villages. These animals also received some form of supplementation with cereal bran and household remains on irregular basis, but the quantity consumed by each animal is not available. Separate shelters are provided for household goats and sheep.

Data collection and analysis

The parameters of interest were fortnight weight gains and daily faecal outputs. All selected animals were tagged, vaccinated against Peste des Petits Ruminants (PPR) and Pasteurellosis, and dewormed. Initial weights were taken at the onset of the study and repeated every fortnight for a period of 75 days.

Two methods of faecal collection were employed. The first method was to collect the animal's overnight faecal droppings during the first 2 weeks. The overnight faecal droppings from each animal was collected, weighed and recorded by one trained assistant based in each village. The second method which lasted for 65 days involved the use of a faecal collection sac fitted on each animal (Figure 1). The sac is fitted on the animals for 24 hours, removed, faecal droppings collected, weighed and recorded. This process was repeated 3 times per week for 10 weeks duration.

Both freshly voided faecal samples (less than 12 hours old) from the monitored animals and old faecal samples that have been accumulated over three months period were also collected. These samples were oven dried at 100° C for 24 hours to estimate their dry matter, then milled, and stored frozen until analysed for Nitrogen using Kjeldahl method as described by Barbano and Clark (1990); and Phosphorus and Potassium contents using Flame Atomic Absorption Spectroscopy (AAS).

The data on average daily weight gain (ADWG), faecal outputs and their mineral contents were analyzed using descriptive statistics and effects of independent variables (age of sample, animal species, breed, village and districts) on dependent variables (ADWG, faecal outputs, and minerals) analyzed using linear regression models in STATA 11.0® statistical package.

Results

Table 2 shows the mean weights and standard deviations of the goats and sheep by breeds and villages. The crossbred sheep at Dibba Kunda Fulla had the highest initial weight of 25.5 kg, final weight of 32.5kg, and average daily weight gain (ADWG) of 93.3 g, followed by Djallonke sheep with average daily weight gain of 51.7 ± 12g. The lowest average daily weight gain of 12.0 ± 16.0g in Djallonke sheep and 26.7 ± 23.9g in WAD goats were observed in Chamen and Bassik, respectively.

In Table 3 the initial and final weights were significantly lower in WADG goats ($p < 0.05$) than DS and DSS sheep. Although the highest average daily weight gain was observed in DSS sheep (40.0 ± 42.7g) followed by WADG (34.8 ± 22.6g) and DS sheep (25.6 ± 25.9g) being the lowest, there was no significant difference ($p > 0.05$).

Table 4 and 5 show the faecal outputs of monitored sheep and goats. The highest overnight faecal output over a 14 day observation period was observed in DS sheep with 286 ± 52g in Bassik village followed by Dibbakunda Fulla (DKF) with 191 ± 7g, and Chamen with 187 ± 11g. For the 24 hour faecal output using harness/faecal sac, the DSS sheep at Palelei with 551 ± 37g had the highest followed by DS sheep breed with 536 ± 30g in Dibbakunda Fulla, Chamen with 269 ± 13g and Bassik with 238 ± 28g in decreasing order (Table 4). There were also no significant differences ($p > 0.05$) in faecal outputs between villages and districts.

For the overnight faecal output (Table 5), the DS sheep breed had the highest followed by DSS sheep breed and then WADG goats. However, the DSS sheep breed had the highest average 24 hour faecal output followed by DS sheep breed and then WADG.

Tables 6 to 8 show results of the chemical analyses of the 28 selected manure samples.

No significant differences ($p > 0.05$) were observed in Nitrogen, Phosphorus and Potassium contents for both fresh and old

Table 1: Number of study animals by breed and location

Villages	Crossbred rams (DSS)	Djallonke rams (DS)	WADG bucks	Totals by village
Bassik	2	4	5	11
Chamen	0	5	4	9
Dibbakunda Fulla	1	4	1	6
Palelei	7	0	1	8
Totals by breed	**10**	**13**	**11**	**34**

Figure 1: Ram and buck fitted with a faecal collection sac/harness

Table 2: Mean weights and average daily weight gains for the three breeds of sheep and goats by village

Village	Species	Breed	n	Mean initial weight (kg)	Mean final weight (kg)	Mean weight gained (kg)	Average Daily Weight Gain (g)
Bassik	Sheep	DSS	2	18.5 ±0.5	19.8 ± 1.8	1.3 ± 2.3	17 ± 42.0
Bassik	Sheep	DS	4	22.1± 2.9	23.4 ± 4.6	1.3 ± 2.0	16.8 ± 30.9
Bassik	Goat	WADG	5	18.2 ±2.0	20.2 ± 2.5	2.0 ± 1.8	26.7± 29.9
Chamen	Sheep	DS	5	21.3 ±4.3	22.2 ± 4.3	0.9 ± 1.2	12.0 ± 16.0
Chamen	Goat	WADG	4	15.1± 0.8	18.0 ± 2.3	2.9 ± 1.7	39.0 ± 26.2
Dibbakunda Fulla	Sheep	DSS	1	25.5	32.5	7.0	93.3
Dibbakunda Fulla	Sheep	DS	4	22.1 ±1.9	26.0± 1.3	3.9 ± 0.9	51.5 ± 13.9
Dibbakunda Fulla	Goat	WADG	1	18.0	21.5	3.5	46.7
Palelei	Sheep	DSS	7	20.3 ±2.0	23.2± 3.1	2.9 ± 3.2	39.0 ± 45.6
Palelei	Goat	WADG	1	14.0	17.5	3.5	46.7

Table 3: Mean weights and average daily weight gains for the three breeds of sheep and goats

Species	Breed	n	Mean initial weight (Kg)	Mean final weight (Kg)	Mean weight gained (Kg)	Average Daily Weight Gain (g)
Sheep	DS	13	21.8b ± 3.3	23.7d ± 4.1	1.9 ± 1.9	25.6e ± 26.9
Sheep	DSS	10	20.5b ± 2.5	23.5d ± 4.3	3.0 ± 3.2	40.0e ± 44.9
Goat	WADG	11	16.7a ± 2.2	19.3c ± 2.6	2.6 ± 1.7	34.8e ± 23.7

Non similar letters under the same column are significantly different (p<0.05)

Table 4: Average overnight faecal and 24 hour faecal output of sheep and goats in the four villages

Village	Species	Breed	Overnight faecal output		24 hour faecal output	
			n	Daily average (g)	n	Daily average (g)
Bassik	Goat	WADG	5	94 ± 9	5	213 ± 43
Bassik	Sheep	DSS	2	96 ± 22	2	238 ± 28
Bassik	Sheep	DS	4	286 ± 52	4	258 ± 31
Chamen	Goat	WADG	4	173 ± 7	4	264 ± 15
Chamen	Sheep	DS	5	187 ± 11	5	269 ± 13
DKF*	Goat	WADG	1	142	1	391
DKF	Sheep	DSS	1	185	1	538
DKF	Sheep	DS	4	191 ± 7	4	536 ± 30
Palelei	Goat	WADG	1	114	1	516
Palelei	Sheep	DSS	7	161 ± 25	7	551 ± 37

**DKF = Dibba Kunda Fulla*

Table 5: Average overnight and 24 hour faecal output of three breeds of sheep and goats across the four villages

Species	Breed	n	Average overnight faecal output (g)	Average24 hour faecal output(g)
Sheep	DSS	10	151 ± 34	487 ± 129
Sheep	DS	13	218 ± 54	347 ± 128
Goat	WADG	11	129 ± 50	275 ± 85

Table 6: Mineral contents of fresh and old manure regardless of animal species

Manure status	Sample size	Dry Matter (%)	Nitrogen (%of DM)	Phosphorous (% of DM)	Potassium (% of DM)
Fresh	14	49.9a± 10.1	2.3c± 0.5	1.0d± 0.3	0.6d± 0.4
Old	14	82.5b± 13.2	2.0c± 0.5	1.0d± 0.2	0.8d± 0.4

Note: *Different superscripts on the same column are significantly different*

Table 7: Mineral contents of fresh and old manure disaggregated by animal species

Manure status	Animal species	Sample size	Dry Matter (%)	Nitrogen (% of DM)	Phosphorous (% of DM)	Potassium (% of DM)
Fresh	Goat	7	50.0 ± 11.6	2.6 ± 0.5	1.1 ± 0.4	0.8 ± 0.4
Fresh	Sheep	7	49.7 ± 11.1	2.1 ± 0.4	0.9 ± 0.2	0.5 ± 0.3
Old	Goat	7	79.4 ± 16.2	2.1 ± 0.4	1.0 ± 0.2	1.1 ± 0.3
Old	Sheep	7	85.6 ± 9.7	1.9 ± 0.5	0.9 ± 0.2	0.6 ± 0.3

Table 8: Mineral contents of goat and sheep manure

Animal species	Sample size	Dry Matter (%)	Nitrogen (%)	Phosphorous (%)	Potassium (%)
Goat	14	64.7 ± 20.4	2.3a± 0.5	1.0 ± 0.3	0.9c± 0.4
Sheep	14	67.7 ± 21.1	2.0b± 0.4	0.9 ± 0.2	0.6d± 0.3
Derfoer *et al* 2000	50 - 70	2.2-3.7	0.25-1.87	0.88-1.25	

manure, however the Nitrogen contents were significantly higher ($p < 0.01$) than Phosphorus and Potassium contents regardless of manure status and animal species as in tables 6 and 7.

Both Nitrogen and Potassium contents of sheep manure were significantly lower ($p < 0.05$) than that of goat manure, and no significant differences were observed in Phosphorus content across manure status, animal species, villages and district (Table 8). By virtue of goats browsing nature, they could ingest feeds that are richer in protein and some minerals than do sheep which might explain these observed differences. There was also no significant difference in the mineral contents of manure from both sheep breeds ($p > 0.05$).

Discussion

No significant differences were observed in the ADWG between different villages and districts as all the animals were under similar management conditions. The crossbred sheep in Dibba Kunda Fulla village was only one animal and seem to have more Sahelian blood and access to better feed than the other crossbred sheep in Bassik and Palelei villages. This could explain why it had the highest ADWG. This could explain why it had the highest ADWG.

Although no significant differences in ADWG was noticed across the three breeds of small ruminants as in table 3, the crossbred sheep had the highest. This finding is in accordance with the fact that crossbred sheep in most cases grow bigger and faster than both Djallonke sheep and WAD goats. The standard deviations of the ADWG for DSS and DS sheep are higher than their means. This is due to the fact that 2 out of 10 DSS sheep and 2 out of 13 DS sheep lost weight by the end of the monitoring period.

The average daily weight gains observed in this study are less than reported values for small ruminants subjected to fattening process in The Gambia. Although these animals were not under fattening, but such comparison would give an indication of their growth potential. For example Sahelian rams under fattening obtained average daily weight gain of 158.3g, Njie (1997). Another fattening trial involving Djallonke rams resulted to ADWG of 120 grams, Njie (1993). The difference is largely due to the big differences in management and feeding strategies. There is an opportunity for farmers' to maximise ADWG of small ruminants when subjected to fattening regime. Sheep fattening is done through zero grazing on basal groundnut hay diet, supplemented with cereals bran and groundnut cake, whereas animals in this study were grazed on communal pastures and irregular supplementation.

The large variations in weight gains (Tables 2 and 3) could be due to many factors. Even though pastures are abundant, the limitation for optimal growth and weight gain in this study could be ascribed mainly to poor feeding and management practices. The allocated time of herding these monitored animals, about five hours daily, seem to be insufficient for them to get enough nutrients that supports optimal growth. The lower nutrient contents of matured and lignified grasses post flowering which the animals depended on entirely from mid October to end November could also have contributed immensely to the small daily weight gains and even losses by some animals. Younger pastures usually contain more proteins and energy.

Other poor management practices such as improper housing with leaking roofs and muddy floors, exposure to harsh weather elements (strong winds, hot sun and rains particularly for goats), and lack of sufficient and

regular supplementary feeding could also have contributed to the observed variations in weight gains and losses. Age differences could also cause variation in ADWG, but these animals were in the same age bracket. Therefore improving the feeding and management practices for these animals through supplementary feeding with locally available crop residues and by-products, longer grazing periods (7-8 hours), and better housing facilities would certainly offset low daily weight gains.

Based on the observed results, it appears that the average 24 hour faecal output is proportional to the size of the animal. This is further corroborated by the fact that sheep's 24 hour faecal output is significantly ($p<0.05$) higher than goats. Further analysis also showed that DSS sheep breed had significantly ($p<0.05$) higher 24 hour faecal output than both DS and WADG breeds.

The mineral contents observed in the manure samples are very similar to those reported by Derfoer et al. (2000) for goat and sheep (Table 8). They also asserted that nitrogen loss from manure could be high especially under aerobic conditions where ammonia volatilization can remove up to 60% of its total Nitrogen content. This could also happen under village conditions where manure is piling up in open pens with large aeration.

The amount of faeces produced by an animal depends on the quantity of feed consumed as well as its digestibility. For example if a small ruminant weighing 25 kg consumes 3.2% of its body weight in dry matter daily, 292 kg (25 kg x 365 days/annum x 0.032) dry matter per annum, with a dry matter digestibility of 60%, this animal will produce approximately 117 kg (0.6 x 292kg) dry faeces annually, Derfoer *et al.,* (2000). The quantity of manure can be further increased by extending the period that the animals are held at their housing facilities and also applying litter on the ground. Therefore small ruminant faeces should be valued as a good potential source of organic manure for use in crop and vegetable production.

Impact

Small ruminant production is very vital in the socio-economic activities of small scale subsistence farmers in rural areas of The Gambia. Small ruminants are one of the main commodities that enhance household food security through the provision of food and income from sales to farmers.

Under the low input production systems which characterises the traditional management system of livestock, the average daily weight gain of small ruminants as found in this study is generally very low. The feeding regime and general animal husbandry practice adopted by these farmers was found to be grossly inadequate to support optimal growth rate of monitored bucks and rams during this study period.

In view of this study finding, more work need to be done to sensitize and support farmers to make positive changes in the feeding and management of small ruminants in order to optimise the average daily weight gains of bucks and rams. Such changes would give rise to larger number of bucks and rams reaching maturity within a shorter period, thus producing big enough animals that could fetch higher selling prices.

Crossbred sheep appear to have a better growth rate and faecal droppings than pure Djallonke rams and West Africa Dwarf goats, however their susceptibility to many endemic diseases makes them less suitable candidate for the low-input traditional system. Preference should be given to the Djallonke and West Africa Dwarf Goats breeds that are more adapted to the low-input production system. However, crossbred sheep could be recommended for market-oriented livestock farmers who would put in more resources to fatten these animals for profit making commercial venture.

Conclusion

In conclusion, the DSS sheep breed grew faster and produced more faecal output than both DS sheep and WADG breeds. It is also the best source of small ruminant

organic manure for farmers' crop fields and horticultural gardens. Based on findings from previous sheep fattening trials, this practice could generate more income from sales of well fattened rams during the Islamic feast of ram sacrifice.

It is recommended that further participatory studies like this should build in a controlled study to get more precise data, because lot of data may not be precisely and accurately collected from participatory studies of this nature. More studies should also be undertaken to investigate the effects of feed supplementation with locally available feed resources such as dual purpose cowpea and groundnut on daily weight gains of small ruminants as well as manure quality and their effects on leguminous crop production.

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References

Barbano DM, Clark JL, 1990. Kjeldahl method for determination of total nitrogen content of milk: collaborative study. Journal - Association of Official Analytical Chemists, 73 (6): 849-859.

Derfoer T, Budelman A, Toulmin C, Carter SE, 2000. Chapter 3: Sources and flows of nutrients in farming. In Managing soil fertility in the tropics. Building common knowledge: participatory learning and action research, Eds., Derfoer T, Budelman A, Toulmin C and Carter SE, ISBN: 90-6832-128-5, pp207.

Jaitner J, Sowe J, Secka-Njie E, Dempfle L, 2001. Ownership pattern and management practices of small ruminants in The Gambia - implications for a breeding programme. Small Ruminant Research, 40(2): 101-108.

Njie M, 1997. Assessment of Feeding Strategies for fattening Ram Lambs using Groundnut hay Supplemented with Sesame Seed Cake and Rice bran in The Gambia. Department of Livestock Services, Abuko, The Gambia.

Njie M, 1993. Economic Assessment of Feeding Strategies for Fattening Ram Lambs Using Sesame Cake in The Gambia. Department of Livestock Services, Abuko, The Gambia.

Osaer S, Goossens B, Kora S, Gaye M, Darboe L, 1999. Health and productivity of traditionally managed Djallonke sheep and West African dwarf goats under high and moderate trypanosomosis risk. Veterinary Parasitology, 82 (2): 101-119

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CBPP $T_1$44/T_1SR VACCINE INDUCED IMMUNE RESPONSE IN VACCINATED CATTLE

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Abstract

The Gambia experienced a sudden epidemic outbreak of Contagious bovine pleuropneumonia (CBPP) in cattle in August 2012 after its last reported cases in 1971. The objective of this study was to monitor the immunological response in terms of antibody detection in vaccinated cattle against CBPP using freeze-dried live attenuated T144 or T1SR strains.

Blood samples were collected from 136 cattle 2 days before vaccination, 135 cattle 2 weeks post vaccination, and 114 cattle at 3 months post-vaccination. The extracted serum samples were tested for the presence of antibodies against Mycoplasma mycoides subsp mycoides Small Colony variant (MmmSC) using IDEXX Contagious Bovine Pleuropneumonia enzyme immunoassay Antibody Test Kit.

Results show that the proportion of cattle with detectable antibodies against CBPP antigens were 15% (9 – 22%), 67% (59 – 75%), and 28% (20 – 37%) at 2 days before vaccination, 2 weeks post vaccination, and 3 months post vaccination, respectively. The proportion of animals with detectable antibodies post-vaccination was significantly higher ($p < 0.05$) than pre-vaccination stage. The seroprevalence of CBPP in the monitored cattle before vaccination was 15% (9 – 22%).

Based on the results obtained, it could be concluded that the vaccinated animals have responded well to the vaccination. Assuming that CBPP vaccine efficacy could be associated to detection of antibodies 2 weeks post-vaccination, then this vaccine's efficacy could be in the range of 59 - 75%. In order to prolong the protection of vaccinated animals, it is recommended that animals should be re-vaccinated 12 months post-vaccination. A longer and more robust longitudinal study involving more animals should be undertaken to determine CBPP vaccine efficacy under local conditions.

Keywords: Contagious bovine pleuropneumonia, cattle, ELISA, The Gambia

RÉPONSE IMMUNITAIRE INDUITE PAR LE VACCIN $T_1$44/T_1SR CONTRE LA PPCB CHEZ LES BOVINS VACCINÉS

Résumé

La Gambie a connu une flambée épizootique soudaine de pleuropneumonie contagieuse bovine (PPCB) en août 2012, alors que le dernier cas signalé sur son territoire remontait à 1971. L'objectif de cette étude était de surveiller la réponse immunologique en termes de détection d'anticorps chez les bovins vaccinés contre la pleuropneumonie contagieuse bovine, en utilisant les souches vivantes atténuées lyophilisées T144 ou T1SR.

Des échantillons de sang ont été prélevés sur 136 bovins 2 jours avant la vaccination, sur 135 bovins 2 semaines après la vaccination, et sur 114 bovins 3 mois après la vaccination. Les échantillons de sérum extrait ont été testés pour rechercher la présence d'anticorps contre la variante de la petite colonie Mycoplasma mycoides subsp mycoides (MmmSC) en utilisant le kit d'épreuves d'anticorps du dosage immuno-enzymatique IDEXX pour la péripneumonie contagieuse bovine.

Les résultats montrent que la proportion de bovins ayant des anticorps détectables dirigés contre des antigènes PPCB était de 15% (9-22%), 67% (59-75%) et 28% (20-37%), respectivement à 2 jours avant la vaccination, 2 semaines après la vaccination et 3 mois après la vaccination. La proportion d'animaux ayant des anticorps détectables après la vaccination était significativement plus élevée ($p < 0,05$) par rapport au stade de pré-vaccination. La séroprévalence de la PPCB dans les troupeaux suivis avant la vaccination était de 15% (9-22%).

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Sur la base des résultats obtenus, on peut conclure que les animaux vaccinés ont bien répondu à la vaccination. En supposant que l'efficacité du vaccin PPCB pourrait être associée à la détection des anticorps 2 semaines après la vaccination, alors l'efficacité de ce vaccin pourrait être de l'ordre de 59 à 75%. Dans la perspective de prolonger la protection des animaux vaccinés, il est recommandé que les animaux soient revaccinés à 12 mois post-vaccination. Une étude longitudinale plus longue et plus exhaustive portant sur un plus grand nombre d'animaux devrait être menée afin de déterminer l'efficacité du vaccin contre la PPCB dans les conditions locales.

Mots-clés : pleuropneumonie contagieuse bovine ; bovins ; ELISA ; Gambie

Introduction

Contagious bovine pleuropneumonia (CBPP) or lung sickness, is an insidious pneumonic disease of cattle caused by Mycoplasma mycoides subspecies mycoides Small Colony variant (MmmSC) and it is one of the major diseases affecting cattle in Africa (Amanfu, 2009). CBPP is a respiratory disease characterised by pneumonia and serofibrinous pleurisy. The usual form of this disease is acute but chronic forms are frequent, particularly in endemic regions. Hyperacute forms, with a high mortality rate, can be seen at the beginning of outbreaks in newly infected regions.

CBPP impacts animal health and poverty of livestock-dependent people through decreased animal productivity, reduced food supply, and the cost of control measures. It is a barrier to trade in many African countries and this reduces the value of livestock and the income of many value chain stakeholders. Its presence also poses a constant threat to CBPP-free countries and creates costs in terms of the measures necessary to ensure the exclusion of disease (Jores *et al.,* 2013).

The epidemiology of the disease in Africa is dominated by four factors, namely: cattle are the only species affected, there is no reservoir in wild animals, clinical cases or chronic carriers are the usual sources of infection, through direct contact, and cattle movements play a very important role in the maintenance and extension of the disease (Thiaucout *et al.,* 2004).

CBPP was last reported in The Gambia in 1971, but its four decade long history of absence was broken by reports reaching the Department of Livestock Services in August 2012 of a suspected outbreak of cases in some villages within Niamina Dankunku District, Central River Region South. The suspected CBPP outbreak was confirmed by isolation of Mycoplasma mycoides subsp mycoides Small Colony variant (MmmSC) from lungs and lymph nodes of seropositive cattle in September 2012.

Following the laboratory confirmation of an outbreak of CBPP in Central River and Upper River Regions and the subsequent follow up assessment mission to the country by the Crises Management Centre - Animal Health of the Food and Agricultural Organisation (FAO), the government of The Gambia through the office of the Minister of Agriculture declared a National Animal Health Emergency with effect from Wednesday 8th November 2012. Emergency preparedness plan to contain the disease outbreak was prepared and it included the temporarily suspension of cattle movement and completion of a national mass cattle vaccination campaign against CBPP by April 2013.

The objective of this activity was to monitor the immunological response of ITC cattle herds located in Niamina East District to vaccination against CBPP using freeze-dried live attenuated T144 or T1SR strains CBPP vaccines certified by PANVAC Quality Control. It specifically determined the proportions of animals with detectable antibodies prior to vaccination, 2 weeks and three months post-vaccination.

Materials and Methods

Sample collections

Three field missions to the four ITC herds (2 bull herds and 2 heifer herds) located in Sambelkunda and Touba villages in Niamina East District, Central River Region South, were undertaken on 13th March, 30th March and 28th June 2013, respectively. Three sets of

blood samples were collected at 2 days before vaccination against CBPP, 2 weeks and 3 months post vaccination, respectively. Three hundred and eighty five (385) blood samples were collected through the external jugular vein of monitored cattle, allowed to coagulate under cold chain, and then spin at a speed of 2500 RCF for 5 minutes. The clear serum samples were transferred to labelled cryotubes, and stored at -20° Celsius until tested.

Monitored cattle

The number of cattle sampled for the first, second and third sampling covered 136 (76 heifers and 60 bulls), 135 (73 heifers and 62 bulls), and 114 (63 heifers and 51 bulls) cattle, respectively. These cattle are of N'Dama breed within the age range of one to four years. These study animals graze in the communal rangelands alongside other resident and transhumant cattle. They are tethered at the holding ground at night and herded during day time. The animals were vaccinated using reconstituted freeze-dried live attenuated bacterial vaccine produced using T144 or T1SR strains of Mycoplasma mycoides subsp. mycoides Small Colony (MmmSC). It is estimated that each field dose contains at least 107 viable Mycoplasma organisms which initiate development of immunity in cattle two weeks post vaccination that could last for about one year (NVI CBPP vaccine Manual). One millilitre of the reconstituted vaccine was given to each cattle subcutaneously on the dorsolateral side of the neck of each vaccinated cattle.

Laboratory test

The 385 serum samples were tested using IDEXX Contagious Bovine Pleuropneumonia enzyme immunoassay Antibody Test Kit for the detection of antibodies directed against Mycoplasma mycoides subsp. mycoides biotype SC (MmmSC) in individual bovine serum samples. The test procedure, result calculations and interpretation was done according to the English version of the Test Kit's manual. Samples with 'percentage of inhibition' greater than or equal to 50% were considered Positive for presence of MmmSC Antibodies.

Table 1: Percent serum samples with detectable antibodies against Mycoplasma mycoides organism

Samples	**Pre-vaccination stage**			**Two weeks post-vaccination stage**			**Three months post-vaccination stage**		
Category	No.	No. +ve	Percentage (95% confidence interval)	No.	No. +ve	Percentage (95% confidence interval)	No.	No. +ve	Percentage (95% confidence interval)
Females	76	9	11.8% (5.6-21.3)	73	52	71.2% (59.4-81.2)	63	19	30.2% (19.2-43.0)
Males	60	11	18.3% (9.5-30.4)	62	39	62.9% (49.7-74.8)	51	13	25.5% (14.3-39.6)
Total	**136**	**20**	**14.7% (9.2-21.8)**	**135**	**91**	**67.4% (58.8-75.2)**	**114**	**32**	**28.1% (20.1-37.3)**

Results

The proportion of cattle with detectable antibodies against CBPP antigens were 15% (9 – 22%), 67% (59 – 75%), and 28% (20 – 37%) at 2 days before vaccination, 2 weeks and 3 months post vaccination, respectively (Table 1). This appears to show that about one fifth of pre-vaccinated cattle, two thirds of 2 weeks post vaccinated cattle, and one fourth of 3 months post vaccinated cattle had detectable MmmSC antibodies. The detected antibodies prior to vaccination correspond to the level of field exposure of these animals to Mycoplasma organisms. Therefore, the seroprevalence of CBPP in these animals prior to vaccination is 15% (9 – 22%).

Discussion

CBPP is regarded as the second most important disease of cattle in Africa. The disease was eradicated from Europe through drastic slaughter campaigns with quarantine and restriction of cattle movements. CBPP was contained in Australia using these methods combined with vaccination. However, the disease remains endemic in Asia and Africa, where it inhibits livestock farming. In these continents, vaccination is the preferred means of control to reduce incidence until complementary disease control measures can be applied. Vaccination campaign efficiency depends on four main factors: good planning and organisation; well-trained, fully equipped and highly motivated staff; high quality vaccines; and good international co-operation. Presently, systematic and repeated vaccination is the method of choice against CBPP in Africa (Sylla *et al.,* 1995).

Currently, two MmmSC attenuated strains are recommended for CBPP vaccination: T144 and T1SR. They could induce immunity for one year, but T1SR protection is shorter. T144 was attenuated by passaging a mild field strain 44 times in embryonated eggs. This ensured an attenuation of the strain while keeping its immunogenic properties. Strain T1sr is a direct derivative of T144, adapted to streptomycin resistance by four serial passages in growth medium with increasing concentrations of streptomycin. T1SR has no residual virulence but induces an immunity for a shorter period of 6 months (OIE, 2014). Some emergency vaccinations, performed in various countries in the southern part of the African continent apparently met with failure casting doubts on the protection afforded by the T1sr strain (Thiaucourt *et al.,* 2000).

CBPP vaccine strain T144 possesses residual virulence that may vary according to local conditions. Post-vaccinal reactions are characterised by a localised inflammatory reaction that develops at the site of injection (Willems' reaction) as early as 1 week post-injection. In many cases this local reaction subsides naturally but in some instances it may become extensive and lead to the death of the animal if no suitable antibiotic treatment is administered (OIE, 2014).

Assessing the potency of CBPP vaccines in vaccinated animals is not an easy and direct activity. There is no susceptible laboratory animal allowing easy potency evaluation, and no strong correlation between antibody titres after vaccination and actual protection. The only way to control the potency of a vaccine is to perform a natural challenge in the susceptible host by the 'in-contact' method. The potency of the grand parental stock has been assessed. Primo-vaccination with the minimum required dose gave a 40–60% protection rate. Higher protection rates have been obtained after repeated vaccinations (OIE, 2014).

The efficacy of some CBPP vaccines has been investigated by a number of studies. Using pathological index of challenges at 3 and 16 months post vaccination with the current vaccine and a formulation with a buffered preparation that maintains Mycoplasma viability at ambient temperature for a longer time, Nkando *et al.,* (2012) reported protection levels of 77% and 52%, and 62% and 52%, respectively. From another trial, Wesonga (2000) found the following results: at three months post vaccination challenge, the efficacy was 68.2 and 59% for T1SR and T144 vaccines, respectively; while at 15 months post vaccination challenge the efficacy was 80.5 and 95.5% for T1SR and T144 vaccines, respectively in cattle vaccinated

twice, while in cattle vaccinated only once the efficacy was 28.7 and 78.2% for T1SR and T144, respectively.

Immunostimulating complexes (ISCOM) vaccine, prepared from the whole detergent-solubilized cells of MmmSC, has been found capable of inducing strong primary and long lasting secondary antibody responses which persisted for more than a year in cattle (Abusugra *et al.,* 1997).

This study has demonstrated a large increase in the proportion of animals with detectable antibodies against MmmSC organism from 14% pre-vaccination to 67% two weeks post-vaccination, and 28% three months post-vaccination. The proportion of animals with detectable antibodies post-vaccination was significantly higher ($p < 0.05$) than pre-vaccination stage. Although a challenge was not instituted, detected antibodies in the followed animals seems to suggest that the vaccinated animals have been protected to some extent. It is also known that vaccinated animals could be protected even without detecting antibodies in circulation. Furthermore, none of the monitored cattle developed any clinical sign of contagious bovine pleuropneumonia for more than one year post vaccination.

According to the manufacture of this ELISA test kit, the test cannot detect antibodies after three months post vaccination. The steep downward fall in the proportion of animals with detectable antibodies from two weeks to three months post-vaccination appears to corroborate assertions made by the test kit's manufacturer.

Conclusion and recommendation

Based on the results obtained, it could be concluded that the vaccinated animals have responded very well to the vaccination. Assuming that CBPP vaccine efficacy could be associated to detection of antibodies at two weeks post-vaccination, it could be inferred that this vaccine's efficacy is in the range of 59 - 75%. In order to prolong the protection of vaccinated animals, it is recommended that animals should be re-vaccinated 12 months post-vaccination. A longer and more robust longitudinal study involving more animals should be undertaken to determine vaccine efficacy under local conditions.

Acknowledgements

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Impact

Contagious bovine pleuropneumonia (CBPP) is a respiratory disease of cattle characterized by pneumonia and serofibrinous pleurisy with high mortality rate particularly at the beginning of an outbreak in newly affected region. The disease caused 50% mortality among cattle in the epicentre of the disease outbreak in Niamina Dankunku of The Gambia which was confirmed in September 2012. At such a high mortality rate, the disease could kill half of the cattle population in The Gambia estimated at 398,472 heads if left uncontrolled.

This disease was largely eradicated in Europe and Australia through slaughtering, quarantine, restriction of cattle movement, and vaccination. It is still endemic in Africa and Asia. Restriction of cattle movement and mass cattle vaccination campaigns were the strategies used in The Gambia to contain the outbreak. Current CBPP vaccines in use have varying efficacy rates under different countries. Use of PANVAC certified vaccines ensures higher chance of vaccine efficacy. Based on this study's findings, it is recommended that antibodies against M. Mycoides subsp. mycoides are monitored in vaccinated animals at strategic periods post-vaccination.

References

Abusugra, I., Wolf, G., Bölske, G., Thiaucourt, F., & Morein, B. (1997). ISCOM vaccine against contagious bovine pleuropneumonia (CBPP) 1. Biochemical and immunological haracterization. Veterinary Immunology and Immunopathology, 59(1-2), 31–48. http://doi.org/10.1016/S0165-2427(97)00067-6

Amanfu, W. (2009). Contagious bovine pleuropneumonia (lung sickness) in Africa. The Onderstepoort Journal of Veterinary Research, 76(1), 13–17.

Jores, J., Mariner, J. C., & Naessens, J. (2013). Development of an improved vaccine for contagious bovine pleuropneumonia: An African perspective on challenges and proposed actions. Veterinary Research, 44(1). http://doi.org/10.1186/1297-9716-44-122

Nkando, I., Ndinda, J., Kuria, J., Naessens, J., Mbithi, F., Schnier, C., … Wesonga, H. (2012). Efficacy of two vaccine formulations against contagious bovine pleuropneumonia (CBPP) in Kenyan indigenous cattle. Research in Veterinary Science, 93(2), 568–573. http://doi.org/10.1016/j.rvsc.2011.08.020

OIE. (2014). Contagious bovine pleuropneumonia. In OIE Terrestrial Manual 2014 (pp. 1–16).

Sylla, D., Litamoi, J., & Rweyemamu, M. M. (1995). Vaccination strategies for contagious bovine pleuropneumonia in Africa. Revue Scientifique et Technique (International Office of Epizootics), 14(3), 577–592.

Thiaucourt, F., Yaya, A., Wesonga, H., Huebschle, O. J., Tulasne, J. J., & Provost, A. (2000). Contagious bovine pleuropneumonia. A reassessment of the efficacy of vaccines used in Africa. Annals of the New York Academy of Sciences, 916, 71–80. http://doi.org/10.1111/j.1749-6632.2000.tb05276.x

Thiaucout F.; Van Der Lugt, J. J. and Provost, A. (2004). Contagious bovine pleuropneumonia. In R. C. Coetzer, J.A.W.; Tustin (Ed.), Infectious disease of livestock (Vol. 3 2nd). Cape Town: Oxford University Press.

Wesonga H.O., T. F. (2000). Experimental Studies on the Efficacy of T1sr and T1/44 Vaccine Strains of Mycoplasma mycoides Subspecies mycoides (Small Colony) against a Field Isolate Causing Contagious Bovine Pleuropneumonia in Kenya - Effect of a Revaccination. Revue Elev. Med. Vet. Paystrop, 53(4), 311–318.

HONEYBEE FORAGE, BEE VISITATION COUNTS AND THE PROPERTIES OF HONEY COLLECTED FROM DIFFERENT AGRO-ECOLOGICAL ZONES OF UGANDA

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Abstract

The aim of the survey was to document honeybee forage plants and asses honeybee visitation counts on different forage plants and properties of honey from selected agro-ecological zones of Uganda. In order to achieve the objectives of the study, a survey of the apiaries and beekeepers was done by selecting fifteen bee farmers with established colonies per agro-ecological zone. A vegetation survey of about two kilometers radius of each apiary for bee forage plants was conducted. The preferred forage plants were established by questionnaires and independent field observations on plants that where visited by honeybees. Samples of honey were collected from apiaries in the selected agro-ecological zones for laboratory analyses. Specifically, honey water content, sugars, pH, acidity and colour were analysed in the laboratory.

The results indicate that honeybees in the different agro-ecological regions had diverse honeybee forage sources. A total of forty six plant species belonging to twenty families were identified as honeybee forage sources. Although, the Eastern and Lake Victoria agro-ecological zones had the greatest number of honeybee forage plant species that were visited by honeybees during the study period, other agro-ecological zones equally had honeybee forage sources that can support the beekeeping industry. Honeybee visitation counts on forage plants during the different times of the day varied significantly in some forage species and not in others. Honeybee visitation counts on forage plants in the different agro-ecological zones did not vary significantly in all cases except between Eastern and Northern agro-ecological zones in the late afternoon. The chemical properties of honey (water, pH acidity and sugar) varied among the agro-ecological zones but in all cases met the UNBS and international standards. From this study, I recommend that beekeepers should plant more honeybee forage plants or crops that can act as sources of forage in cases where the natural honeybee forage has been cut down. In addition, bee farmers should be trained on proper honey harvesting and processing techniques so that they can ensure no contamination of honey.

PLANTES FOURRAGERES MELLIFERES, NOMBRE DE VISITES D'ABEILLES ET PROPRIETES DU MIEL RECUEILLI DANS DIFFERENTES ZONES AGRO-ECOLOGIQUES DE L'OUGANDA

Resume

Le but de l'enquête était de documenter les plantes fourragères mellifères et d'évaluer le nombre de visites d'abeilles mellifères sur les différentes plantes fourragères et les propriétés du miel dans certaines zones agro-écologiques de l'Ouganda. Dans l'optique d'atteindre les objectifs de l'étude, une enquête sur les ruchers et les apiculteurs a été réalisée après la sélection de quinze apiculteurs ayant des colonies établies par zone agro-écologique. Une étude de la végétation d'un rayon d'environ deux kilomètres pour chaque rucher a été réalisée en vue d'une documentation des plantes fourragères mellifères. Pour déterminer les plantes fourragères préférées, l'étude a utilisé des questionnaires et des observations indépendantes sur le terrain là où les plantes étaient visitées par les abeilles. Des échantillons de miel ont été recueillis dans les ruchers dans les zones agro-écologiques sélectionnées pour des analyses en laboratoire. Plus précisément, la teneur en eau, les sucres, le pH, l'acidité et la couleur du miel ont été analysés au laboratoire.

Les résultats indiquent que les abeilles mellifères des différentes régions agro-écologiques avaient diverses sources mellifères. Quarante-six espèces de plantes au total, appartenant à vingt familles, ont été identifiées comme sources mellifères. Bien que les zones agro-écologiques de l'Est et du lac Victoria aient

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le plus grand nombre d'espèces de plantes mellifères visitées par les abeilles pendant la période d'étude, les autres zones agro-écologiques avaient également des sources de plantes mellifères à même de soutenir l'industrie de l'apiculture. Le nombre de visites sur les plantes mellifères au cours des différentes périodes de la journée a montré une variation significative chez certaines espèces fourragères et pas d'autres. Les nombres des visites des abeilles sur les plantes fourragères dans les différentes zones agro-écologiques n'ont pas varié de manière significative dans tous les cas, à l'exception des zones agro-écologiques de l'Est et du Nord vers la fin de l'après-midi. L'on a noté une variabilité des propriétés chimiques du miel (eau, pH, acidité et sucre) selon les zones agro-écologiques, mais dans tous les cas elles satisfaisaient aux normes UNBS et aux normes internationales. De cette étude, je recommande que les apiculteurs plantent plus d'espèces fourragères mellifères ou des cultures susceptibles de servir de sources de mellifères dans les cas où les plantes fourragères mellifères naturelles ont été réduites. En outre, les apiculteurs devraient être formés aux techniques appropriées de récolte et de traitement du miel afin qu'ils puissent éviter toute contamination du miel.

Introduction

The beekeeping industry in Africa is relatively undeveloped with heavy reliance on trapping swarms from wild populations to constitute the managed stocks and breeding is seldom done (Dietmann *et al.,* 2009). In addition, beekeeping is widely practiced on small scale by rural people and as groups supported by developmental projects. However, some large scale enterprises with more than 250 hives have been established in Zambia, South Africa and Tanzania. Managed honey bees amount to 14-18 million hives in the whole of Africa (Dietmann *et al.,* 2009).

In Uganda, beekeeping is an important activity mainly in rural areas where it provides a supplementary source of income for many rural households. It was estimated that beekeepers in Uganda had over 750,000 beehives in 2008 and of which 65% were colonized with an estimated honey production of 2,600 MT per year (MAAIF, 2012). In the national live stock census carried out in 2008, Uganda bureau of statistics reported that, Northern Uganda has the highest production (640MT), while the central region has the least (85metric tones). In terms of districts, the census showed that Yumbe has the highest production with (130MT), Nakapiripirit (88MT), Pader (81.3MT), Arua (78.5MT), Moroto (70.6MT), Amuru (57MT), Oyam (47.8MT), Nyadri (44MT), Nebbi (42.6MT), Apac (40.6MT), and Lira (40.5MT). The common types of bee hives used are local hives, accounting for 87.3% of the total hives, the rest being Kenya top bar and langstroth hives.

Bees depend more or less entirely on flowers for resources, both in terms of their own energy needs as well as for provision of the nest larvae. The attractiveness of nectar to the honey bees is probably most affected by its taste (Adler, 2000), but may also be affected by odor (Farina *et al.,* 2007) and color (Thorp *et al.,* 1975). The taste of nectar is dominated by a high sugar concentration. Several studies have attempted to define whether honey bees prefer nectars that are rich in sucrose or hexose. Wykes (1952) showed that honey bees prefer a sugar ratio of 1:1:1 (sucrose/fructose/glucose) over a pure sucrose solution. Odors guide bees toward flowers and may affect their attractiveness (von Frisch, 1967). The volatile components of nectar, including phenolic compounds, form particular odor bouquets (Anklam, 1998). Some of these compounds are more attractive to honey bees than others (Jay, 1986; Winston and Slessor, 1993). Colors also affect the attractiveness of flowers (von Frisch, 1967; Giurfa *et al.,* 1995), and nectar may contribute to their visual display (Thorp *et al.,* 1975). To meet these needs, bees can travel up to a maximum of 5983 m from their habitat while foraging (Hagler *et al.,* 2011).

Honey is a natural sweet subsistence produced by honeybees from nectar of blossoms modified, and stored in honey combs (NHB 1996). Honey is an important energy food used as an ingredient in manufactured foods such as cereal-based products for its sweetness, colour, flaour, caramelization and viscosity. It is medicinal because of its valuable properties that include hygroscopic nature, antimicrobial and antioxidant properties (White et al. 1975).

Under Codex Alimentarius, the following are the specific standards for honey: sugar content, not less than 65%; moisture content, not more than 21%; sucrose content, not less than 5%; water insoluble solid for honey which is not pressed 0.1% and pressed honey 0.5%; mineral content, not more than 0.6%; acidity, not more than 40 milliequivalents acid per 1000 grams; diastase activity, more than 3; hydroxymethylfurfural content, not more than 80mg/kg. Honey should not have any objectionable flavor, aroma or taint absorbed from foreign matter during its processing and storage and does not contain plant toxins in any amount which may constitute a hazard to health (UNBS, 2005). The color of honey varies from almost colourless to nearly black according to its botanical source and conditions of processing and storage it has under gone. The colour of honey is a key honey quality parameter and is very important in marketing and determining the end use (Kugonza, 2009). Generally, light coloured honeys are preferred to darker honeys. The consistency can be fluid, viscous or partly to entirely crystallized and the flavor and aroma vary, but are derived from the plant origin (UNBS 2005). The current study investigated honeybee forage plants and assessed honeybee visitation counts on different forage plants and properties of honey from selected agro-ecological zones of Uganda.

The problem statement

The beekeeping industry in Uganda provides a source of livelihood to many people including vulnerable groups. The honeybee production heavily relies on available forage both for pollen and nectar. The properties of honey are influenced by the nectar source (forage) and the marketing of honey of a known nectar source adds more value to the honey since it can be blended as coffee, eucalyptus etc depending on the nectar source. There is little empirical information on sources of honeybee forage and honey properties in Uganda. In addition, the properties of honey from different agro-ecological zones have not been assessed despite its importance in designing honey marketing strategies. The current study documented honeybee forage plants and assessed honeybee visitation counts on different forage plants and properties of honey from selected agro-ecological zones of Uganda. Since honey is produced from nectar collected by honeybees from plants, understanding the most visited forage plants in that area can help in selecting forage for planting. Furthermore, since the forage source also influences the quality of honey, an understanding of the bee forage source is of paramount importance. This information will contribute to the planning and implementation of the promotion of honeybee forage that is preferred by honeybees and that produces honey with good qualities.

General objective

To investigate honeybee forage behavior (forage plants and assess honeybee visitation on different forage plants) and properties of honey from selected agro-ecological zones of Uganda.

Specific objectives

1. To identify species foraged on by honeybees in selected agro-ecological zones of Uganda.
2. To determine honeybee visitation counts on different forage plants in selected agro-ecological zones of Uganda.
3. To determine the properties of honey collected from selected agro-ecological zones of Uganda.

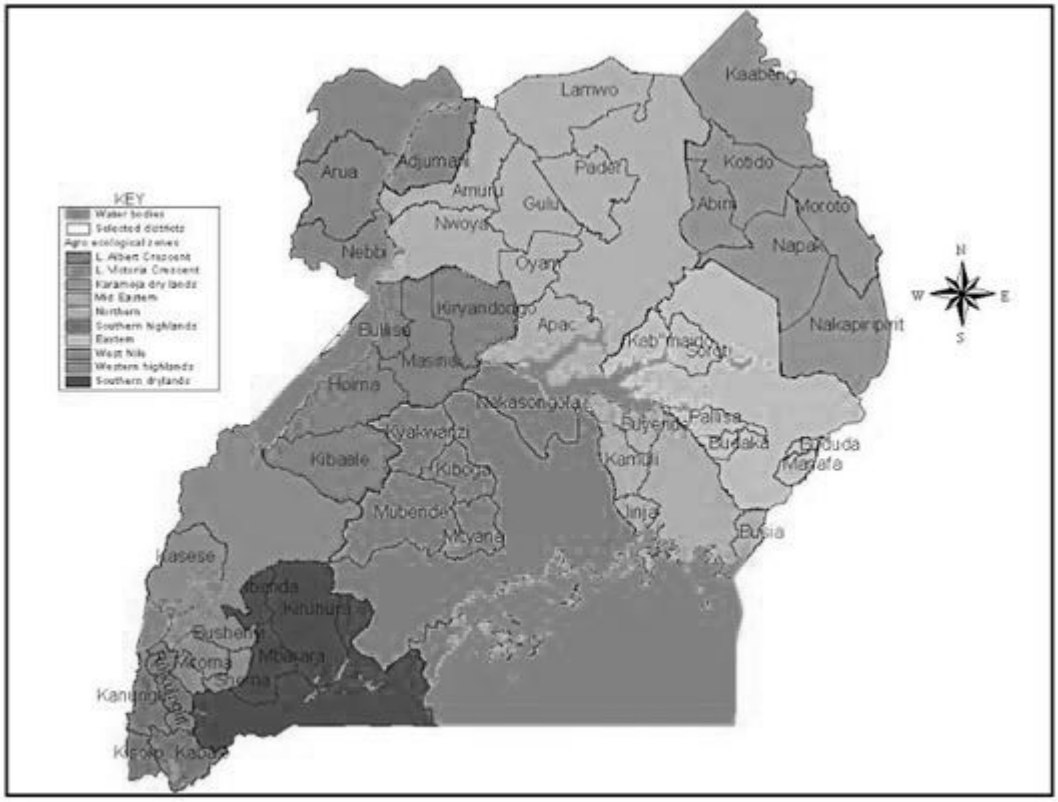
Null hypotheses

1. The visitation times on different honeybee forage plants by bees do not vary across different agro-ecological zones of Uganda.
2. The properties (sugar content, pH, acidity, moisture content and colour) of honey from different agro-ecological zones of Uganda do not vary significantly.

Materials And Methods

Study Area

This study was conducted in nine agro-ecological zones of Uganda.



The agro-ecological zones of Uganda and the districts surveyed

Research design

A survey of the apiaries and beekeepers was done by selecting fifteen bee farmers with established colonies per agro-ecological zone. A vegetation survey of about two kilometers radius of each apiary for bee forage plants was conducted. The Apis mellifera preferred forage plants were established by questionnaires and independent field observations on plants that where visited by honeybees. Samples of honey were collected from apiaries in the selected agro-ecological zones for laboratory analyses.

Identifying honeybee forage plants in different agro-ecological zones of Uganda

At least one district was selected per agro-ecological zone for the study. Within each district, at least two parishes known for beekeeping were visited and beekeepers chosen at random for interviews. The beekeepers were interviewed on bee forage plants and surveys conducted within approximately 2-3 kilometer radius of their apiaries to establish honeybee forage plants visited by the bees. Where plants could not be easily identified in the field, specimens were collected and transported to the herbarium at Makerere University for identification.

Honeybee visitation on different forage plants

A total number of 46 honeybee forage plants species, from 20 families within 2 km of selected apiaries in the different districts and agro-ecological zones were visited three times during the day (morning 7-10am; afternoon 12-2pm; and late afternoon 4-5pm) for counts on the numbers of honeybees visiting the plants for nectar/pollen. This was done during the major flowering season (March to July). Honeybees visiting the bee forage plant were counted for five minutes during each counting session and recorded. This was done during the rainy season when most plants had flowered.

Determining the properties of honey collected from different agro-ecological zones of Uganda

Collection of honey samples

In each of the nine agro-ecological zones honey samples were collected from three or four districts, in which four to six sub counties where sampled from. Two to three honey samples from different hives were sampled from each beekeeper labeled for easy identification during laboratory work. All the honey samples collected were strained using a mesh of similar size (400 micron) before being taken to Makerere University Faculty of Agriculture Laboratory for chemical analysis.

The determination of honey colour

The colour of honey samples collected was determined using the Tintometer apparatus. In the procedure, honey was spread over white halon block and viewed over reflected light. The colour of the honey samples was measured using a Lovibond Model E tintometer for language colour from red to violet according to the Lovibond system. A sample of honey to be viewed was placed in position by spreading it over the slide so that it could be seen in the left hand field of the viewing tube. The triangular knobs which control the coloured filters were slided towards the right adjusting the red, yellow and blue in correct proportion until a perfect colour match was obtained. Values of the slides were recorded as shown in the indicator aperture in the slotted plate.

Determination of honey moisture content

The refractometric method (Chataway, 1932) and revised by Wedmore (1955) was used to estimate the moisture content of honey. A refractometer (Model RHB-90) (FAO, 1990) was used to measure the moisture content of honey samples. This method was selected because it offers the most rapid and convenient way of estimating the water content of honey (Kugonza, 2009). For each honey sample, a drop of honey was placed on refractometer and then directed to light before focusing and taking the readings.

Determination of the Honey pH (Acidity)

The pH of each honey sample was determined following the procedure of AOAC (1999) described by Kugonza (2009). In the procedure, 10 g of each honey sample was dissolved in 75 ml of carbon dioxide free water in a 250 ml beaker. The solution was then stirred magnetically with the electrode immersed for pH monitoring. The resultant solution was then titrated with 0.05 M NaOH, and the titre value recorded. Immediately, 10 ml of 0.05 M NaOH was added and the solution back titrated with 0.05 M Hcl to a pH of 8.5. A blank that was similar to the set-up but lacking honey was included. Free and total acidity were computed as follows:

Free acidity (milli-equivalents of acid kg-1 of honey) =

$$\frac{(\text{sample titre-blank}) \times 0.05 \times 1000}{10}$$

Lactone =

$$\frac{(10 - \text{titre of 0.05 M HCl}) \times 0.05 \times 1000}{10}$$

Total acidity =
(free acidity + lactone (milli-equivalents of acid kg^{-1} of honey)

Analysis of sugar content of honey

Determination of reducing sugar content

The Fehling method was used to determine the reducing sugar content of honey (SBRC, 2000). Five (5) ml of Fehling's solution was titrated at boiling point against a solution of honey (2 gm of honey in 250 ml of distilled water) using methylene blue as an internal indicator. The percentage of reducing sugars present was calculated using the following formula:

% Sugar =

$$\frac{\text{mg Sugar/ml Fehling's}}{\text{Titre}} \times \text{vol. Fehling's} \times \frac{\text{Total vol. Sugar} \times 1}{\text{wt sample } 10}$$

Determination of the total sugar content

The Fehling method described by Lane and Eynon (1923) and reviewed by SBRC, (2000) was used to determine the total sugar

content of honey. The honey sample was prepared by dissolving 2 g of the honey sample in 250 ml distilled water. Then, 50 ml of this solution was boiled in a water bath for 10 min, and left to cool. Five (5) ml of HCl was added to each. The mixture was neutralised with sodium hydroxide. Then the honey solution was titrated against 5 ml Fehling's solution. Total sugars were calculated using the following formula:

$$\% \text{ Sugar} = \frac{\text{mg Sugar/ml Fehling's}}{\text{Titre}} \times \text{vol. Fehling's} \times \frac{\text{Total vol. Sugar} \times 1}{\text{wt sample } 10}$$

Non reducing sugars present in Honey

The non reducing sugars were obtained by subtracting the reducing sugars from the total sugars.

Data analysis

SPSS Program (version 17) was used to analyse the data. One way Analyses of Variance (ANOVA) and the Scheffe post-hoc were used to compare honeybee visitation counts on flowers of selected plant species during different times of the day. In addition, the same analysis was used to compare the honey parameters measured: moisture content, sugar and pH contents from different agro-ecological zones sampled.

Results

Honeybee forage plants in different agro-ecological zones of Uganda

Forty six plant species belonging to twenty families were identified as honeybee forage sources by bee farmers and through direct field observation in the study sites (Table 1).The Eastern and LakeVictoria agro-ecological zones had the greatest number (41 species) of honeybee forage plant species that were visited by bees during the study period. On the other hand, the lowest number of honeybee forage plants were recorded in Southern highlands (19 species) followed by Karamoja dry lands (23 species).

Honeybee Visitation Counts on Different Forage Plants

Influence of time of day on honeybee visitation counts on forage plants

Honeybees visited forage plants as early as 6:30 am. However, observations for this study where limited to morning (7-10am), afternoon (12-2pm) and late afternoon (4-5pm) to ensure that the different times of the day were sampled. There were variations on the number of honeybees that visited the same honeybee forage plants at the different times of the day in some cases. For example, honeybee visitation counts of Acacia seyal, Ananas comosus, Canna indica, Curcurbita pepo, Mannithot glaziovii and Persea americana were significantly different during different times of the day (Table 2). Specifically, for Acacia seyal, there were statistically significant differences between groups (times of the day) as determined by one-way ANOVA ($F(2,51) = 4.169$, $p = 0.021$). A Scheffe post-hoc test revealed that only morning (7-10am) visitation counts were statistically significantly higher than evening (4-5pm) (morning; 17.06 ± 0.56, $p = 0.036$) compared to evening (14.67 ± 0.61, $p = 0.036$). There were no statistically significant differences between the morning and afternoon; afternoon and evening ($p > 0.05$). Similarly, for Acacia hockii, there were statistically significant differences between groups (times of the day) as determined by one-way ANOVA ($F(2,48) = 3.372$, $p = 0.043$). A Scheffe post-hoc test did not reveal any statistically significant differences (morning; 17.65 ± 0.57: afternoon; 17.59 ± 0.46: evening: 15.94 ± 0.55; $p > .0.05$). Furthermore, for Ananas comosus, there were statistically significant differences between groups (times of the day) determined by one-way ANOVA ($F(2,102) = 32.682$, $p < 0.01$). A Scheffe post-hoc test revealed that morning (7-10am) visitation counts were significantly higher than evening (4-5pm) (morning; 3.57 ± 0.19, $p < .01$) compared to evening (1.74 ± 0.85, $p < 0.01$). The visitation counts in the afternoon were also significantly higher than those in the evening (afternoon; 3.09 ± 0.15, $p < 0.01$) compared to evening (1.74 ± 0.85, $p = 0.01$).

Counts of bee visitation rates on Canna indica showed that there were

Table 1: Honeybee forage plants reported by farmers and observed in the different agro-ecological zones of Uganda

Forage plants Scientific name	**Agro-ecological zones**								
	Eastern	*Karamoja drylands*	*Lake-Albert crescent*	*Lake Victoria crescent*	*Northern*	*Southern_drylands*	*Southern highlands*	*West Nile*	*Western highlands*
Agava sisalana	X	X	X	X	X	X	X	X	X
Sensevieria guineensis	X	X	X	X	X	X		X	X
Ananas comosus	X	X	X	X	X	X	X	X	X
Canna indica	X	X	X	X	X	X	X	X	X
Sorghum bicolor	X	X	X	X	X	X	X	X	X
Saccharum officinaarum	X	X	X	X	X	X	X	X	X
Zea mays	X	X	X	X	X	X	X	X	X
Sorghum arundinaceum	X	X	X	X	X			X	X
Musa paradisiaca	X	X	X	X	X	X	X	X	X
Elaeis guineensis				X					
Mangifera indica	X	X	X	X	X	X	X	X	X
Anacardium occidentale	X	X			X				X
Ceiba pentandra	X				X				
Carica papaya	X	X	X	X	X	X	X	X	X
Helianthus annus	X	X	X	X	X	X	X	X	X
Bidens pilosa	X	X	X	X	X	X	X	X	X
Guizotia scrabra	X	X	X	X	X	X	X	X	X
Vernonia amygdalina	X	X	X	X	X	X	X	X	X
Curcurbita pepo				X	X	X	X		
Lageenaria sphaerica	X	X	X	X	X	X	X	X	X
Ricinus communis	X	X	X		X	X	X	X	X
Mannihot esculenta	X	X	X	X	X	X	X	X	X
Mannihot glaziovii	X	X	X	X	X	X	X	X	X
Persea americana	X	X	X	X	X	X	X	X	X
Tamarindus indica	X	X	X	X	X			X	X
Albizia zygia	X	X	X	X	X	X	X	X	X
Acacia seyal	X	X	X	X	X			X	X
Acacica senegal	X	X	X	X	X			X	X
Acacica sieberiana		X	X	X	X	X	X	X	X
Acacica hockii	X	X	X	X	X	X	X	X	X
Acacica mellifera	X	X	X	X	X	X	X	X	X
Acacica polyacantha	X	X	X	X	X	X	X	X	X
Phaseolous vugaris	X	X	X	X	X	X	X	X	X

Forage plants Scientific name	**Agro-ecological zones**								
	Eastern	*Karamoja drylands*	*Lake-Albert crescent*	*Lake Victoria crescent*	*Northern*	*Southern_drylands*	*Southern highlands*	*West Nile*	*Western highlands*
Crotalaria juncea	X	X	X	X	X			X	X
Glycine max	X	X	X	X	X	X	X	X	X
Gossypium hirsutum	X	X	X	X	X	X	X	X	X
Hibiscus aponeurus	X	X	X	X	X			X	X
Milica excelsa	X	X	X	X		X	X	X	X
Psidium guajava	X	X	X	X		X	X	X	X
Callistemon citriunus	X	X	X	X	X	X	X	X	X
Eucalyptus camldulensis	X	X	X	X	X	X	X	X	X
Eucalyptus citridora	X	X	X	X	X	X	X	X	X
Eucalyptus maculate		X	X	X	X	X	X	X	X
Sesamum indicum					X				
Coffea camephora	X	X	X	X	X	X	X	X	X

Where X means present

statistically significant differences between groups (times of the day) determined by one-way ANOVA ($F(2,87) = 23.81$, $p < 0.01$). A Scheffe post-hoc test revealed that morning (7-10am) visitation counts were significantly higher than evening (4-5pm) (morning; 4.23 ± 0.40, $p < 0.01$) compared to evening (2.60 ± 0.32, $p < 0.01$). The visitation counts in the afternoon were also significantly higher than those in the evening (afternoon; 4.03 ± 0.36, $p < 0.05$) compared to evening (2.60 ± 0.32, $p < 0.05$). The visitation counts in the morning and afternoon were not statistically different.

Counts of bee visitation on Curcurbita pepo showed that there were statistically significant differences between times of the day determined by one-way ANOVA ($F(2,48) = 9.09$, $p < 0.01$). A Scheffe post-hoc test revealed that only morning (7-10am) visitation counts were significantly higher than evening (4-5pm) (morning; 4.18 ± 0.29, $p < 0.01$) compared to evening (2.65 ± 0.29, $p < 0.01$). The visitation counts in the afternoon were not significantly different from those in the evening (afternoon; 3.41 ± 0.15, $p > 0.05$) compared to evening (2.65 ± 0.29, $p > 0.05$). The visitation counts in the morning and afternoon were not statistically significant. Analyses of counts of bee visitation on Mannihot glaziovii showed that there were statistically significant differences between times of the day determined by one-way ANOVA ($F(2,66) = 7.61$, $p < 0.01$). A Scheffe post-hoc test revealed that morning (7-10am) visitation counts were statistically significantly higher than evening (4-5pm) (morning; 5.87 ± 0.49, $p < 0.01$) compared to evening (3.83 ± 0.25, $p < 0.01$). The visitation counts in the afternoon were significantly higher than those in the evening (afternoon; 5.04 ± 0.34, $p < 0.05$) compared to evening (3.83 ± 0.25, $p < 0.05$). The visitation counts in the morning and afternoon were not statistically different. Counts of bee visitation rates on Persea americana showed that there were statistically significant differences between times of the day determined by one-way ANOVA ($F(2,72) = 3.42$, $p < 0.05$). A Scheffe post-hoc test revealed that morning (7-10am) visitation counts were statistically significantly higher than evening (4-5pm) (morning; 7.68 ± 0.39, $p < 0.05$) compared

Table 2: Bee Visitation counts of different forage plants at different times

Plant species	Mean number of bees visiting different forage plants at different times of the day			p value
	7-10 am	12-2 pm	4-5pm	
Acacia seyal	17.05 ±056	16.72 ±.071	14.66 ± 0.61	<0.05
Acacia hockii	17.64 ±0.56	17.58± 0.46	15.94 ± 0.54	<0.05
Acacica polyacantha	15.34±0.84	15.68±0.92	13.90±0.97	>0.05
Acacica senegal	19.21± 0.54	18.47± 0.67	17.34± 0.69	>0.05
Acacica sieberiana	16.27 ± 1.30	16.13± 1.32	15.00± 1.23	>0.05
Agava sisalana	3.77±0.18	3.58±0.23	3.12 ±0.13	=0.05
Albizia zygia	16.00 ¬± 0.83	15.95± 0.75	14.09± 0.63	>0.05
Anacardium occidentale	12.33± 0.08	11.66± 0.76	10.66 ± 0.76	>0.05
Ananas comosus	3.57 ± 0.19	3.08± 0.14	1.74± 0.14	< 0.05
Bidens pilosa	11.44± 1.09	11.33 ± 0.98	9.88± 0.93	>0.05
Callistemon citriunus	13.95 ± 0.88	13.25 ± 0.98	12.16± 0.96	>0.05
Canna indica	4.23 ± 0.40	4.03± 0.36	2.60±0.32	<0.05
Carica papaya	5.17 ± 0.80	5.58 ±0.90	4.70± 0.83	>0.05
Ceiba pentandra	6.41± 0.90	6.00± 1.05	6.083± 1.01	>0.05
Coffea camephora	10.33 ± 1.46	9.58 ± 1.23	9.41 ± 1.23	>0.05
Crotalaria juncea	5.27 ± 0.40	5.45 ± 0.51	3.68 ±0.38	>0.05
Curcurbita pepo	4.17 ± 0.28	3.41 ± 0.14	2.64 ± 0.29	<0.05
Eucalyptus spp	14.58± 0.53	14.32 ±0.53	12.98 ± 0.49	>0.05
Glycine max	4.22 ± 0.40	4.27± 0.49	3.66 ±0.56	>0.05
Gossypium hirsutum	6.42 ± 0.57	6.85 ± 0.633	5.14 ± 0.55	>0.05
Guizotia scrabra	8.26 ±0.87	7.57 ± 0.79	6.73 ±0.77	>0.05
Helianthus annus	11.5 ± 0.86	10.9 ± 0.95	9.83 ± 0.93	> 0.05
Hibiscus aponeurus	6.71 ± 0.54	6.42 ± 0.61	4.78 ± 0.59	=0.05
Lageenaria sphaerica	6.44 ±1.39	6.00 ± 1.11	5.22 ±1.46	>0.05
Mangifera indica	15.28± 0.93	15.5 ± 1.13	14.14 ± 0.95	> 0.05
Manithot esculenta	6.26 ±0.39	5.84 ± 0.30	4.94 ± 044	=0.05
Manithot glaziovii	5.86 ± 0.48	5.04 ± 0.34	3.82 ± 0.24	<0.05
Milica excelsa	4.60 ± 0.60	4.73 ± 0.52	4.20 ± 0.45	>0.05
Musa paradisiaca	10.0 ± 0.73	9.43 ± 0.75	8.68 ±0.81	>0.05
Persea americana	7.68 ± 0.39	7.32 ± 0.41	6.24 ± 0.41	<0.05
Phaseolous vugaris	5.76 ± 0.68	5.23 ± 0.47	4.76 ± 0.49	>0.05
Psidium guajava	5.40 ± 0.38	5.32 ±0.39	4.68 ± 0.40	>0.05
Ricinus communis	6.57 ± 0.33	6.51 ± 0.34	5.93 ± 0.34	>0.05
Saccharum officinaarum	10.9 ± 0.86	10.2 ± 0.92	8.07 ± 0.85	>0.05
Sensevieria guineensis	4.63 ± 0.39	4.36 ± 0.34	2.80 ± 0.30	<0.05
Sesamum indicum	7.15 ± 1.31	6.46 ± 1.24	6.23 ± 1.37	>0.05
Sorghum arundinaceum	9.71 ± 1.64	10.14 ± 1.66	9.00 ± 1.57	>0.05
Sorghum bicolor	11.6 ± 0.80	11.1 ± 0.83	9.73 ± 0.73	>0.05

Plant species	Mean number of bees visiting different forage plants at different times of the day			p value
	7-10 am	12-2 pm	4-5pm	
Tamarindus indica	6.16 ± 0.54	5.87 ± 0.41	4.70 ± 0.33	=0.05
Vernonia amygdalina	6.33 ± 1.14	6.73 ± 0.87	5.26 ± 0.84	>0.05
Vigna unguiaculate	3.42 ± 0.29	3.57 ± 0.36	2.42 ± 0.52	>0.05
Zea mays	6.47 ± 0.67	6.00 ± 0.64	4.61 ± 0.54	>0.05

Table 3: Mean honey bees visitation counts in different agro-ecological at a specific time

Time	agro-ecological zones	Mean number of bees at different times	P value
7-10am	Eastern	8.51±0.36	>0.05
	Karamoja drylands	9.21±0.71	
	Lake Albert crescent	9.22±0.57	
	Lake Victoria crescent	9.63±0.49	
	Northern	9.76±0.57	
	Southern drylands	9.02±0.83	
	Southern highlands	7.90±o.94	
	West Nile	9.79±0.67	
	Western Highlands	8.96±0.50	
12-2pm	Eastern	8.16±0.36	>0.05
	Karamoja drylands	8.98±0.72	
	Lake Albert crescent	8.87±0.54	
	Lake Victoria crescent	9.56±0.51	
	Northern	9.55±0,54	
	Southern drylands	8.40±0.83	
	Southern highlands	7.74±0.96	
	West Nile	9.50±0.70	
	Western highlands	8.64±0.50	
4-5pm	Eastern	6.68±0.34	<0.01
	Karamoja drylands	7.86±0.69	
	Lake Albert crescent	7.48±0.49	
	Lake Victoria crescent	8.24±0.48	
	Northern	9.41±0.56	
	Southern drylands	7.17±0.75	
	Southern highlands	7.16±0.94	
	West Nile	8.17±0.64	
	Western highlands	7.50±0.48	

to evening (6.24 ± 0.41, $p < 0.05$). The visitation counts in the afternoon were not significantly different from those in the evening (afternoon; 7.32 ± 0.41, $p > 0.05$) compared to evening (6.24 ± 0.41, $p > 0.05$). Similarly, the visitation counts in the morning and afternoon were not statistically significant.

Analyses of the bee visitation rates on Sensevieria guineensis showed that there were statistically significant differences between times of the day determined by one-way ANOVA ($F(2,87) = 8.09$, $p < 0.01$). A Scheffe post-hoc test revealed that morning (7-10am) visitation counts were statistically significantly higher than evening (4-5pm) (morning; 4.63 ± 0.39, $p < 0.05$) compared to evening (2.80 ± 0.30, $p < 0.05$). The visitation counts in the afternoon were also significantly higher than those in the evening (afternoon; 4.37 ± 0.34, $p < 0.05$) compared to evening (2.80 ± 0.30, $p < 0.05$). However, the visitation counts in the morning and afternoon were not statistically different.

In some species, the visitation counts were not significantly different during the different times of the day (Table 2).

Variation in honeybee visitation counts on forage plants in different agro-ecological zones Generally, honeybee visitation counts did not vary significantly in the morning and afternoon. However, honeybee visitation counts on forage plants varied significantly in the late afternoon (4-5 pm) among the different agro-ecological zones (Table 3). A Mann-Whitney U test revealed that only the mean visitation counts of the Eastern and Northern agro-ecological zones in the late afternoon were significantly different (mean for Eastern 6.68±0.34 and mean for northern 9.41±0.56, $p<0.05$).

Discussion

Several studies have revealed that honeybee forage may vary from one region to another due to differences in topography, climate and farming practices (Haftom, 2013; Amsalu, 2006; FAO 1990). This is in agreement with results of the current study which indicate that forty six plant species belonging to twenty families were identified as honeybee forage sources by bee farmers and through field observations in the study sites. Some agro-ecological zones (specifically the Eastern and Lake Victoria) had the greatest number of honeybee forage plant species that were visited by honeybees during the study period. This may be attributed to the environmental factors such as fertile soils and reliable rainfall within the two agro-ecological zones which favour plant species diversity. On the other hand, the lowest number of honeybee forage plants was recorded in Southern highlands and Karamoja dry lands. The low number of honeybee forage species in Karamoja region can be attributed to the harsh weather conditions that do not favour many plant species. Low forage species numbers in the southern highlands may be attributed to high human settlement on the land leading to clearance of natural vegetation and replacement with monocultures of few plant species.

The time of day and forage species have been highlighted to have positive correlations with the visitation counts on forage plants. In the current study, the honey bee visitation counts on Acacia seyal, Acacia hochii. Ananas comous, Canna indica, Curcurbita pepo Manihot glaziovii, Persea Americana and Sensevieria guineensis were found to be significantly high in the morning than other times of the day. This may be attributed to the honeybees' activity which is related to environmental temperature. Honeybees are more active in foraging when it is not too hot hence more visits to the forage plants during the morning hours. As the day gets hotter, they become less active. In the hot afternoons many bees were seen on water containers collecting water to cool the temperatures of the hive. Studies have shown that certain insect species have precise daily times of foraging activity (Stone et al. 1999). This might be attributed to the cycle of flower opening. For example, the anthers of Acacia senegal ripen and burst open releasing the polyads during mid-day to mid afternoon and the pollen dehiscence cycle starts at 11.00 am. Honeybee visits may follow this cycle in

order for them to collect maximum amounts of pollen from these plants.

On the other hand, honeybee visitation counts on the majority of forage plants were not significantly different during different times of the day. This may be attributed to the study sites lying within the tropics where temperatures do not vary greatly during the day. The honeybees therefore can forage throughout the day due to suitable temperatures for their foraging activities.

As expected, the findings of this study show that there were no significant differences in the honeybee visitation counts of forage in the different agro-ecological zones during the time different times of the day. I attribute this to tropical climatic conditions in all the study sites which allow the honeybees to visit forage plants throughout the day.

Conclusions

From this study, I can draw the following conclusions:

- Honeybees in the different agro-ecological regions have diverse honeybee forage sources. A total of forty six plant species belonging to twenty families were identified as honeybee forage sources. Although some agro-ecological zones (specifically the Eastern and Lake Victoria) had the greatest number of honeybee forage plant species that were visited by honeybees during the study period, the other agro-ecological zones equally have honeybee forage sources that can support the beekeeping industry.
- Honeybee visitation counts on forage plants during the different times of the day varied significantly in some forage species and not in others.
- Honeybee visitation rates of forage plants in the different agro-ecological zones did not vary significantly in all cases except between Eastern and Northern agro-ecological zones in the late afternoon.

Recommendations

- Beekeepers should plant more honeybee forage plants or crops that can act as sources of forage in cases where the natural honeybee forage has been cut down
- More research on the pollen in the honey collected from the different agro-ecological zones should be carried out in order to understand the key sources of nectar in the different regions of the country.
- Research on honeybee foraging behavior such as honeybee dance language in the different habitat types should be conducted.

References

Alqarni, A.S. (2006) Tolerance of summer temperature in imported and indigenous honeybee Apis mellifera L. races in central Saudi Arabia. Saudi Journal of Biological Sciences 13 (2): 123-127

Amsalu, B. (2006). Seasonal intensity of flowering and pollen-forage selectivity by honeybees, Apis mellifera bandasii in central highlands of Ethiopia. Proceedings of the 14th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, September 5–7, 2006. Part II: Technical Papers. , Addis Ababa, Ethiopia. pp 3-10.

Anita, M., Sivaram, V. and Jayaramappa, K.V. (2012). Influence of bee attractants on pollination and yield parameters in Guava (Psidium guajava L.). The International Journal of Plant Reproductive Biology 4(1):41-46.

Biesmeijer, J.C. and Ermers, M.C.W. (1999). Social foraging in stingless bees: how colonies of Melipona fasciata choose among nectar sources. Behavioral Ecology and Sociobiology, 46(2), 129–140.

Contreras, H.L., Goyret, J., Arx, M.v., Pierce, C.T., Bronstein, J.L., Raguso, R.A. and Davidowitz, G. (2013). The effect of ambient humidity on the foraging behavior of the hawkmoth Manduca sexta. Journal of Comparative Physiology, 199(11),1053-63.

Crane, E. (1990). Bees and Beekeeping: Science, Practice and World Resources. Ithaca, NY: Comstock Plushing Associates.

DCA (Dan Church Aid), (2010). Climate change and adaptation strategies in the Karamoja sub region.http://www.danchurchaid,org/content/download/77266/642633/file/Uganda%20

climate%20report,%20may%202010.pdf

FAO (Food and Agriculture Organisation of the United Nations) (1990). Beekeeping in Africa. Agricultural Services Bulletin 68/6 Food and Agriculture Organisation of the United Nations Rome.ISBN 92-5-102794-3.96pp.

Haftom G, Tesfay Z, Murutse G and Estifanos A (2013). Seasonal honeybee forage availability, swarming, absconding and honey harvesting in Debrekidan and Begasheka Watersheds of Tigray, Northern Ethiopia. Livestock Research for Rural Development. Volume 25, Article #61. Retrieved December 9, 2014, from http://www.lrrd.org/lrrd25/4/haft25061.htm.

Hossam, F.A., Ahmad, A.A. and Abdelsalam, A.M. (2012). Tolerance of two honey bee races to various temperature and relative humidity gradients. Environmental and Experimental Biology, 10, 133–138.

Joshi, N.C. and Joshi, P.C. (2010). Foraging Behaviour of Apis Spp. on Apple Flowers in a Subtropical Environment. New York Science Journal, 2010;3(3)

Kamanyire, M. (2000). Sustainability Indicators for Natural Resource Management and Policy, Viewed,04/09/2013,http://www.fao/fileadmin/user_upload/kagera/resource/ug_nrm_overview_paper.pdf

Kajobe, R., Agea, J.G., Kugonza, D.R., Alioni, V., Otim, S.A., Rureba, T. and and Marris, G. (2009). National Beekeeping Calendar, Honeybee Pest and Disease Control Methods for Improved Production of honey and other hive products in Uganda, Final Project Report Submitted to National Agricultural Research Organisation (NARO), Entebbe Uganda.

Kirk, R.S. and Sawyer, R. (1991). Pearson's Composition and Analysis of Foods. 9th Edition. Longman Group, UK England.

Lane, K.K. and David, R.T. (2006). Environmental and genotypic effects on Russian-Hybrid and Italian Honey Bee (Apis mellifera) (Hymenoptera: Apidae) foraging behavior. Environmental entomology, 35(6), 1610-1616(7).

Mattu, V. K., Hem, R. and Thakur, M.L. (2012). Foraging behavior of honeybees on apple crop and its variation with altitude in Shimla hills of western Himalaya, India. International journal of science and nature, 3(1), 296-301.

NEMA (National Environment Management Authority), (2009). Uganda; Atlas of our changing environment, viewed 4/09/2013, http://www.content.yudu.com/Alfdtr/UAtlas/resources/60.htm.

Okello, J. (2010). Morphological and Nutritional characteristics of Tarmarindicus Indica (Linn) fruits in Uganda. mac.ac.ug/documents/makfiles/theses/okellojasper.pdf.

START (Global Change System for Analysis, Research and Training), (2009). Assessment of the impact of climate change and climate variability on crop production in Uganda, viewed 4/09/2013, http://www.start.org/download/gec08/wasige-final.pdf

Stone, G.N., Gilbert, F., Willmer, P., Potts, S., Semida, F. and Zalat, S. (1999). Windows of opportunity and the temporal structuring of foraging activity in a desert solitary bee. Ecol. Entomol. 24:208–221. doi:10.1046/j.1365-2311.1999.00181.

Tirado, R., Simon, G. and Johnston, P. (2013). Bees in Decline: A review of factors that put pollinators and agriculture in Europe at risk. Greenpeace Research Laboratories Technical Report (Review). Greenpeace International, Ottho Heldringstraat 5, 1066 AZ Amsterdam, the Netherlands.

Tripath, H. (2011). Beekeeping and agricultural productivity: Role of beekeeping with indigenous bee Apis cerana in crop production. Under the mango tree. Pp 12-47. Retrieved from http://utmt.in/wp-content/uploads/2013/09/Research-Final-compressed-version-july-2012.pdf

EPIZOOTIOLOGY OF NEWCASTLE DISEASE IN TWO LIVE BIRD MARKETS IN IBADAN, SOUTH WESTERN NIGERIA

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Abstract

Newcastle disease (ND) is a devastating viral disease of poultry worldwide. This study was therefore undertaken to understand the role of live bird markets (LBMs) in the epizootiology of ND in Nigeria. A structured questionnaire was administered to poultry dealers and cloacal swab sampling of live birds in two LBMs in Ibadan was done. Three hundred pooled swab samples were collected from five different apparently healthy bird species sold in the markets over a period of one month. Virus isolation was performed in embryonated chicken eggs using the cloacal swab samples. Twenty one isolates of ND virus were obtained from four of the five bird species. Isolation rates of 24.4%, 20.0%, 18.8%, and 18.5% were obtained from chickens, ducks, guinea fowls, and pigeons respectively. There was no statistically significant difference ($p > 0.05$) in the ND virus isolation rates from the different bird species. Market level isolation rates of 16.3% and 25.5% were found in Molete and Shasha respectively which was also not statistically different ($p > 0.05$). The results show that LBMs are important sources of transmission and threat of NDV to the commercial and backyard poultry farms in Nigeria. There is therefore need for surveillance for ND virus and effective control strategies against its continuous circulation in the country.

Keywords: Newcastle Disease, Live Bird Markets, Virus Isolation, Epizootiology, Ibadan Nigeria

EPIZOOTIOLOGIE DE LA MALADIE DE NEWCASTLE DANS DEUX MARCHÉS D'OISEAUX VIVANTS À IBADAN DANS LE SUD-OUEST DU NIGERIA

Résumé

La maladie de Newcastle (MNC) est une maladie virale dévastatrice de la volaille, présente dans le monde entier. Cette étude a donc été réalisée pour comprendre le rôle des marchés d'oiseaux vivants dans l'épizootiologie de la MNC au Nigeria. Un questionnaire structuré a été administré à des vendeurs de volailles, et des échantillons d'écouvillons cloacaux d'oiseaux vivants dans les deux marchés d'oiseaux vivants à Ibadan ont été prélevés. Trois cents écouvillons composites ont été recueillis à partir de cinq différentes espèces d'oiseaux apparemment sains vendus aux marchés sur une période d'un mois. L'isolement du virus a été réalisé dans des œufs de poule fécondés, en utilisant des échantillons d'écouvillons cloacaux. Vingt-et-un isolats de virus de la MNC ont été obtenus dans quatre des cinq espèces d'oiseaux. Des taux d'isolement de 24,4%, 20,0%, 18,8%, et 18,5% ont été obtenus respectivement pour les poulets, les canards, les pintades et les pigeons. L'on n'a pas noté de différence statistiquement significative ($p> 0,05$) entre les taux d'isolement du virus de la MNC dans les différentes espèces d'oiseaux. Des taux d'isolement de 16,3% et de 25,5% au niveau des marchés ont été enregistrés respectivement à Molete et Shasha, lesquels taux étaient également non statistiquement différents ($p> 0,05$). Les résultats montrent que les marchés d'oiseaux vivants sont d'importantes sources de transmission et de menace de MNC pour les exploitations avicoles commerciales et artisanales au Nigeria. Il est donc nécessaire de mettre en place des stratégies de surveillance et de contrôle efficace de la circulation continue du virus de la MNC dans le pays.

Mots-clés : maladie de Newcastle ; marchés d'oiseaux vivants ; isolement du virus ; épizootiologie ; Ibadan, Nigeria

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Introduction

The poultry production system in Nigeria is generally comprised of both commercial and free range poultry estimated at 183 million (Fadiga *et al.,* 2013). The latter accounts for the majority (over 90%) of Nigeria poultry population. Most commercial and free ranged poultry end up in Live Bird Markets (LBMs) where multiple species of poultry are sold. These LBMs exist in most urban settings and villages across Nigeria. Birds entering these markets are sourced from different vendors who move from farm to farm and villages in search of birds for purchase. The markets serve as source of live birds for sale and slaughtered for consumption poultry for those who patronize them. In some cases, live chickens bought from the market are taken home and mixed with the existent flock (Nwanta *et al.,* 2008).

ND virus also referred to as avian paramyxovirus type I (APMV-1) is in the order Mononegavirales, a member of the Paramyxoviridae family and genus avulavirus (ICTV, 2012). Based on clinical signs, three different pathotypes of ND strains have been identified namely: lentogenic (low virulence), mesogenic (intermediate virulence) and velogenic (high virulence) (OIE, 2009). The disease infects wide host range in which all orders of birds have been reported (Alexander, 1997). ND has been identified as a serious constraint to poultry production in both commercial and rural poultry industry (Nwanta *et al.,* 2008). The first reported and confirmed outbreak of the disease in Nigeria was in Ibadan, South Western part of the country in 1952 (Hill *et al.,* 1953). Since then, ND has become enzootic in Nigeria and controlling it has been a major problem both in vaccinated and unvaccinated backyard and commercial poultry settings (Manchang *et al.,* 2004). Results of sero-prevalence studies on the infection of NDV in different parts of Nigeria has been reported (Adu *et al.,* 1986; Oyekunle *et al.,* 2006; Musa *et al.,* 2009; Nwankiti *et al.,* 2010). Recently, Ibu et al. (2009) reported on the prevalence of ND in caged and wild birds. However, limited information exists on the virological and epizootiological studies in LBMs. This study is therefore aimed at determining the role of LBMs in the epizootiology of the disease in the study area.

Materials and Methods

Description of study area

This study was carried out in Ibadan, Oyo State (figure 1) where the disease was first reported (Hill *et al.,* 1953). The state is located in south western Nigeria and covers an area of 28, 454 km2 with a population of 5, 591, 589 people based on the 2006 census. The state has 33 local government areas (LGAs) with Ibadan as the capital. Located on Latitude 8oN and Longitude 4oE, the state is bounded to the north by Kwara, on the east by Osun, the south by Ogun, on the west partly by Ogun and Republic of Benin. The study was conducted in two major LBMs in Ibadan metropolis, namely: Molete (MO) and Shasha (SA). The two markets were chosen due to the number of poultry stock holding and volume of sales. The markets are opened to customers for 10 – 12 hours daily.

Data collection

A questionnaire designed to identify possible risk factors for the maintenance of NDV in the LBMs, sanitation practices and types and sources of birds sold was administered simultaneously with cloacal sample collection from birds on sale in the markets.

Sample collection and processing

A total of 300 cloacal swab samples were collected five time from randomly selected apparently healthy live chickens, ducks, pigeons, guinea fowls, and turkeys in the two LBMs from March to April, 2008. No sample from sick or dead birds was included in the study. Swabs from same bird species and epidemiological units were pooled (n=3) in virus transport medium (VTM) given a total of 100 pools. The VTM was made of glycerol and phosphate-buffered saline (1:1 v/v) containing antibiotics concentration of 10,000 U/ml penicillin, 10 mg/ml streptomycin, 0.25 mg/ml gentamycin and 0.0125 mg/ml amphotericin B adjusted to a

Figure 1: Map of Nigeria showing Oyo state (Ibadan) where samples were collected and states from the Northern region where poultry supplies are made to Shasha market.

final pH of 7.2 (OIE, 2004). The samples were transported to the laboratory in an ice cold box and stored at -80°C until analyzed.

The specimens were processed for virus isolation at the Avian Virus Research Laboratory (AVRL) of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The cloacal swabs were thawed, vortexed and a sterile forceps was used to remove the swab sticks. Suspension of the swab contents in each vial was centrifuged at 3000 rpm for 10 mins and the supernatant used as the inoculum.

Virus Isolation and Identification

Virus isolation was performed in 9-11 day-old specific NDV antibody negative embryonated chicken eggs according to standard protocol (OIE, 2004). Briefly, embryonating eggs were inoculated with 0.2ml of the inoculum. Inoculated eggs were incubated at 37oC and examined daily by candling for evidence of embryo death for 5 days. Embryonic deaths above 24 hours of inoculation were refrigerated and if no mortality was observed, the eggs were chilled at 4oC after the expiration of the incubation period. Allantoic fluid (ALF) from the refrigerated eggs were harvested and tested for haemagglutinating (HA) activity using 10% washed chicken red blood cells. ALF was examined for bacteria contamination by plating on bacteriological medium.

Standard haemagglutination and haemagglutination-inhibition (HI) test was used in identifying NDV using antiserum obtained from the AVRL of NVRI, Vom according to the World Organization for Animal Health diagnostic manual (OIE, 2004).

Statistical Analysis

The percentage (total number of isolate/total number of pooled sample) of isolates recovered was computed. Pearson's chi-squared test was used to test for differences in the number of test positives (isolates recovered) and number of test that were negatives (no isolates) between sampled markets and among bird types. A $p < 0.05$ was considered significant for all tests. All analyses were performed using R statistical software version 3.0.1.

Results

Data from the questionnaires showed that most of the birds sold at Molete market were commercial and free ranged chickens, pigeons, ducks, guinea fowls and turkeys. In addition, animals sold in the market include dogs, rabbits, guinea pigs and cats. Information provided by the poultry sellers/workers showed that most of the birds sold in this market were sourced from commercial poultry farms within and outside Ibadan metropolis mainly Abeokuta, Ijebu, Ogbomoso, and Akure where the birds were raised purposely for meat and/or egg production. Also, birds were routinely supplied to this market by dealers in Shasha market. Similarly, the bird population at Shasha market comprised of free ranged local chickens, pigeons, ducks, guinea fowls, and turkeys. In addition to poultry, the dealers also trade in guinea pigs and rabbits. Most of the birds sold in this market were brought mainly from the north western parts of Nigeria, namely Jigawa, Katsina, Sokoto, Kebbi, and Zamfara states (figure 1). The pooled cloacal swab samples comprised of chickens 41%, ducks 15%, guinea fowls 16%, pigeons 27%, and turkey 1% (Table 1). The samples yielded a total of 21 isolates identified as NDV by the HI test given an isolation rate of 21.0% of cloacal sample pools with detectable NDV from the two markets (Table 1). Of the twenty-one isolates, 8 (16.3%) and 13 (25.5%) were obtained from MO and SA respectively. The isolates were obtained from five different batches of birds sampled during the study period in the two markets (3 times at MO and 2 times at SA).

Based on specie, NDV was isolated from 10 (24.4%) chicken from the 2 markets and 3 (20.0%) isolates from duck samples. Similarly, 3 (18.8%) isolates were obtained from guinea fowls and the pigeon samples yielded 5 (18.5%) isolates while there was none from the turkey samples (Table 1). None of the samples was positive for avian influenza virus (data not shown). Overall, NDV was isolated from 21.0% (21/100) of the pooled samples. There were no significant differences in percentage of NDV isolated between markets ($p = 0.2607$), and among different bird types ($p = 0.9503$) (Table 2).

Table 1: Distribution of samples collected and NDV isolates by Species from Molete and Shasha markets

Species	Molete (MO)		Shasha (SA)		Total pooled samples	Total isolates (%)
	Number of pooled samples	Number of Isolate (s)	Number of pooled samples	Number of Isolate (s)		
Chickens	30	4	11	6	41	10 (24.4)
Ducks	6	1	9	2	15	3 (20.0)
G/Fowl	4	1	12	2	16	3 (18.8)
Pigeons	8	2	19	3	27	5 (18.5)
Turkeys	1	0	0	0	1	0 (0)
Total	**49**	**8**	**51**	**13**	**100**	**21**

Table 2: Chi square test for market and species from Molete and Shasha LBMs in Ibadan, Nigeria

Variables	Number of pooled sample	Number of Isolates	% of Isolate	X^2	p-value
Market					
Molete (MO)	49	8	16.30		
Shasha (SA)	51	13	25.49	1.265	0.2607
Species					
Chickens	41	10	24.39	0.708	0.9503
Ducks	15	3	20.00		
Guinea fowl	16	3	18.75		
Pigeons	27	5	18.52		
Turkeys	1	0	0.00		

Discussion

The overall isolation rate of 21.0% obtained in this study is higher than the 16.0% reported from a similar study in 10 Vietnamese LBMs in 2001 over a two day sampling period (Nguyen *et al.,* 2005) and 3.2% reported from a 5 year surveillance in Eastern China (Liu *et al.,* 2009). A recent study conducted in Republic of Korea found an isolation rate of 0.17% from LBM surveillance over 2 years (Kim *et al.,* 2012). Occurrence of ND outbreak has been reported to be higher during the dry season (October-March) in Nigeria (Manchang *et al.,* 2004; Musa *et al.,* 2009) a period when this study was carried out and may therefore explain the high isolation rate for NDV found in this study.

Comparison of the rate of isolation of NDV between the two markets showed that Shasha market had a higher rate (25.5%) than Molete (16.3%) though statistically insignificant ($p = 0.2607$). The higher rate observed from Shasha could be attributed to the predominance of free range local chickens, ducks, pigeons and guinea fowls from the source where local birds have been reported to harbour NDV by previous workers (Echeonwu *et al.*,, 1993; Nwanta *et al.,* 2008). Data from this study also indicate that Shasha market supplies Molete market and other LBMs in Ibadan and environs with local chickens, pigeons, guinea fowls and ducks. Movements of these live and possibly infected birds for trading or exchange as gifts have been suggested to be a major mode of transmission of the virus (Schelling *et al.,* 1999; Nwanta *et al.,* 2008). The poor and low frequency of decontamination of cages, type of cages used, and stocking of different bird species as revealed by the questionnaire may be responsible for the continuous perpetuation of the virus in the markets.

NDV was isolated from four of the five species of birds sampled in this study. The highest isolation rate of 24.4% was from chickens followed by ducks 20.0%, guinea fowls 18.8% and pigeons 18.5% which was not statistically significant ($p = 0.9503$). Isolation of low-virulence NDV during surveillance of LBMs in the north-eastern U.S with different properties from commonly used commercial vaccine strains have been reported (King and Seal 1997; Seal *et al.,* 2005). Also, isolation of velogenic NDV from apparently healthy free-roaming chickens, ducks, and pigeons as well as dead birds in Nigeria has been reported (Majiyagbe and Nawathe 1981; Adu *et al.,* 1985; Echeonwu *et al.,* 1993). Emergence of virulent strain of NDV due to genomic changes of an endemic lentogenic NDV that circulated for a long time among domestic poultry was reported in Australia (Gould *et al.,* 2001). Ducks have been implicated to harbour the virus in its apathogenic forms and subsequently transmitted to domestic poultry where the virus may undergo mutation to its virulent forms. It is possible that vaccination of chickens with live ND virus that is regularly practiced in commercial poultry may be responsible for subsequent isolation of vaccine virus from

birds when sold to LBMs. The pathotypes of NDV isolates is yet to be determined. The characterization and phylogenetic analysis of the isolates may provide a better understanding of their origin and relatedness to vaccine strains being used in the country.

It can be deduced from the results of this study that NDV is circulating in apparently healthy chickens, ducks, pigeons and guinea fowls in the two LBMs at different times during the study period. Chickens were identified as the major host harbouring NDV in the markets. The result of this study further established the endemicity of NDV in Nigeria as previously reported (Adu *et al.,* 1985; Manchang *et al.,* 2004; Oyekunle *et al.,* 2006) and the continuous isolation of this virus in these LBMs may play a vital role in the maintenance and spread of NDV to commercial poultry and other susceptible hosts in the vicinity. Surveillance of LBMs in the country is therefore advocated for early detection of circulating ND virus and inclusion of the role of LBMs in planning of control strategies against ND in Nigeria.

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References

Adu FD, Edo U, Sokale B, 1986. Newcastle disease: The immunological status of Nigerian chickens. Tropical Veterinarian, 24: 149 – 152.

Adu FD, Oyejide O, Ikede BO, 1985. Characterization of Nigerian strains of Newcastle disease virus. Avian Diseases, 29: 829 – 831.

Alexander DJ, 1997. Newcastle disease and avian paramyxovirus infections. In Diseases of poultry, 10th edn. Eds., Calnek, B.W.; Barnes, H.J.; Beard, C.W.; McDougald, L.R.; Saif YM. Iowa State University Press, Ames, IA, pp: 541–569

Echeonwu GO, Iroegbu CU, Emeruwa AC, 1993. Recovery of velogenic Newcastle disease virus from dead and healthy free-roaming birds in Nigeria. Avian Pathology, 22: 383 – 387.

Fadiga M, Jost C, Ihedioha J, 2013. Financial costs of disease burden, morbidity and mortality from priority livestock diseases in Nigeria. Disease burden and cost–benefit analysis of targeted interventions. In: ILRI Research Report. Volume 33 Visited March 14, 2015 from https://cgspace.cgiar.org/bitstream/handle/10568/33418/ResearchReport_33.pdf?sequence=2.

Gould AR, Kattenbelt JA, Selleck P, Hansson E, Della-Porta A, Westbury HA, 2001. Virulent Newcastle disease in Australia: Molecular epidemiological analysis of viruses isolated prior to and during the outbreaks of 1998-2000. Virus Research, 77: 51 – 60.

Hill HD, Davis OS, Wilde JE, 1953. Newcastle disease in Nigeria. British Veterinary Journal 109: 381 – 385.

Ibu J, Okoye J, Adulugba E, Chah K, Shoyinka S, Salihu E, Chukwuedo A A, Baba S, 2009. Prevalence of Newcastle Disease virus in wild and captive birds in Central Nigeria. International Journal of Poultry Science, 8: 574 – 578.

ICTV, 2012. Virus Taxonomy. In: Ninth report of the International Committee on Taxonomy of Viruses. Eds. A. King, E. Lefkowitz, M. . Adams, and E. B. Carstens London, United Kingdom: Elsevier doi:10.1016/B978-0-12-384684-6.00111-7.

Kim B-YY, Lee D-HH, Kim MS, Jang JH, Lee Y-NN, Park J-KK, Yuk SS, Lee JB, Park SY, Choi IS, Song CS, 2012. Exchange of Newcastle disease viruses in Korea: the relatedness of isolates between wild birds, live bird markets, poultry farms and neighboring countries. Infection, genetics and evolution, 12: 478 – 482.

King DJ., and Seal BS, 1997. Biological and molecular characterization of Newcastle disease virus isolates from surveillance of live bird markets in the northeastern United States. Avian Diseases, 41: 683 – 689

Liu X, Wang X, Wu S, Hu S, Peng Y, Xue F, Liu X, 2009. Surveillance for avirulent Newcastle disease viruses in domestic ducks (Anas platyrhynchos and Cairina moschata) at live bird markets in Eastern China and characterization of the viruses isolated. Avian Pathology, 38: 377 – 391.

Majiyagbe KA, and Nawathe DR, 1981. Isolation of virulent Newcastle disease virus from apparently normal ducks in Nigeria. Veterinary Record, 108: 190.

Manchang T, Abdu P, Saidu L, 2004. Epidemiology and Clinicopathologic Manifestations of Newcastle Disease. Pathologie Infectieuse, 57: 35 – 39.

Musa U, Abdu PA, Dafwang II, Umoh JU, Saidu L, Mera UM, Edache JA, 2009. Seroprevalence , Seasonal Occurrence and Clinical Manifestation of Newcastle Disease in Rural Household Chickens in Plateau State , Nigeria. International Journal of Poultry Science, 8: 200 – 204.

Nguyen DC, Uyeki TM, Jadhao S, Maines T, Shaw M, Matsuoka Y, Smith C, Rowe T, Lu X, Hall H, Xu X, Balish A, Klimov A, Tumpey TM, Swayne DE, Huynh LPT, Nghiem HK, Nguyen HHT, Hoang LT, Cox NJ, Katz JM, 2005. Isolation and characterization of avian influenza viruses, including highly pathogenic H5N1, from poultry in live bird markets in Hanoi, Vietnam, in 2001. Journal of Virology, 79: 4201 – 4212.

Nwankiti O, Ejekwolu A, Ibrahim I, Ndako J, Echeonwu G, 2010. Detection of serum antibody levels against Newcastle Disease in local chickens in Bauchi metropolis, Bauchi State , Nigeria. African Journal of Clinical and Experimental Microbiology, 11: 95 – 101.

Nwanta JA, Abdu PA, Ezema, W, 2008. Epidemiology, challenges and prospects for control of Newcastle disease in village poultry in Nigeria. World's Poultry Science Journal, 64: 119 – 127.

OIE, 2004. "Newcastle disease," in Manual of Standards for Diagnostic Tests and Vaccines (Paris, France), pp: 270—282.

Oyekunle MA, Talabi AO, Okeowo AO, 2006. Serological Status for Newcastle Disease Virus in Unvaccinated Indigenous Chickens in Yewa Division of Ogun State , Nigeria. International Journal of Poultry Science, 5: 1119 – 1122.

Schelling E, Thur B, Griot C, Audige L, 1999. Epidemiological study of Newcastle disease in backyard poultry and wild bird populations in Switzerland. Avian Pathology, 28: 263 – 272.

Seal BS, Wise MG, Pedersen JC, Senne DA, Alvarez R, Scott MS, King DJ, Yu Q, Kapczynski DR, 2005. Genomic sequences of low-virulence avian paramyxovirus-1 (Newcastle disease virus) isolates obtained from live-bird markets in North America not related to commonly utilized commercial vaccine strains. Veterinary Microbiology, 106: 7 – 16.

GROWTH AND REPRODUCTIVE PERFORMANCE OF CAPTIVE GRASS CUTTER (Thryonomys swinderianus TEMMINCK) ON FEEDS SUPPLEMENTED WITH Moringa oleifera LAM.

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Abstract

Feeding trial were conducted in one- tier cage (1.40 x 0.60 x 0.90m) to assess the growth and reproductive performance of grass cutter (Thryonomys swinderianus) on feeds supplemented with Moringa oleifera leaf. Five experimental diets were formulated at 22% crude protein content with 0, 25, 50, 75 and 100% M. oleifera as partial replacement for Groundnut Cake (GNC). Each treatment was replicated twice with 2 T. swinderianus (one male and female) per replicate of average body weight 1290.37±0.25g. Thryonomys swinderianus were fed twice daily to satiation/ad libitum. Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Mortality Rate (MR), Litter Size (LS), Birth Weight (BW) and Survival Rate (SR) were measured. Data were analyzed using descriptive statistics and ANOVA at p = 0.05. Results showed that the T. swinderianus on M. oleifera leaf based diets had higher growth than those on control diet but T. swinderianus fed 100%n inclusion of M. oleifera leaf had significant higher MWG, SGR and FCR of 255.35± 3.12g, 0.03±0.00g and 0.04±0.00 respectively. Highest mortality were recorded in the control (3) while lowest were recorded in 75 and 100% M. oleifera leaf inclusion (0, 0) respectively. Also, 25 and 50% inclusion of M. oleifera leaf recorded lowest litter size (4) while highest (5) were recorded in other treatments. Hundred percent survival rate were recorded in 75 and 100% inclusion of M. oleifera leaf and this were significantly different ($p<0.05$) from the control (40%). Highest birth weight of fawn was recorded in 100% M. oleifera leaf inclusion (135.0 g) and was significantly different ($p<0.05$) from other treatments. These suggest that M. oleifera leaf could be a potential and promising dietary supplementation that would affect growth, and reproductive performance of T. swinderianus in captivity.

Keywords: Thryonomys swinderianus, Animal growth, Moringa oleifera, Reproductive, Experimental diets

PERFORMANCE DE CROISSANCE ET DE REPRODUCTION DES AULACODES CAPTIFS (Thryonomys swinderianus TEMMINCK) SOUMIS A DES REGIMES COMPLETES AVEC Moringa oleifera LAM.

Resume

Un essai alimentaire a été réalisé en cage à un seul niveau (1,40 x 0,60 x 0,90 m) pour évaluer la croissance et la performance reproductive des aulacodes (Thryonomys swinderianus) soumis à une alimentation complétée avec des feuilles de Moringa oleifera. Cinq régimes expérimentaux ont été formulés à une teneur en protéines brutes de 22% avec 0, 25, 50, 75 et 100% de M. oleifera, en remplacement partiel de tourteaux d'arachide (TA). Chaque traitement a été répété deux fois, avec 2 T. swinderianus (un mâle et une femelle) par répétition, d'un poids corporel moyen de 1290,37 ± 0,25 g. Thryonomys swinderianus ont été nourris deux fois par jour à satiété / ad libitum. Le gain pondéral moyen (GPM), le taux de croissance spécifique (TCS), le taux de conversion alimentaire (TCA), le taux de mortalité (TM), la taille de la portée (TP), le poids à la naissance (PN) et le taux de survie (TS) ont été mesurés. Les données ont été analysées à l'aide de statistiques descriptives et l'analyse de variance ANOVA à p = 0,05. Les résultats ont montré que les T. swinderianus nourris au régime à base de feuilles de M. oleifera ont enregistré une croissance plus élevée que ceux soumis au régime témoin, mais les T. swinderianus nourris avec un régime comportant une inclusion de feuilles de M. oleifera de 100% avaient un GPM, un TCS et un TCA significativement plus élevés, respectivement de 255.35 ± 3,12 g, 0,03 ± 0.00g et 0,04 ± 0,00. La plus forte mortalité a été enregistrée

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dans le groupe témoin (3), tandis que le taux de mortalité le plus bas a été enregistré pour l'inclusion de feuilles de M. oleifera (0, 0) de 75 et 100%. En outre, une inclusion de feuilles de M. oleifera de 25 et 50% a conduit à la plus petite taille de portée (4), tandis que la plus grande taille de portée (5) a été enregistrée dans d'autres traitements. Le taux de survie de cent pour cent a été enregistré à une inclusion de feuilles de M. oleifera de 100 et 75%, et il était significativement différent (p <0,05) du traitement témoin (40%). Le poids de naissance le plus élevé du faon a été enregistré pour l'inclusion de feuilles de M. oleifera à 100% (135,0 g) et était significativement différent (p <0,05) pour les autres traitements. Ceci laisse penser que la feuille de M. oleifera pourrait être un complément alimentaire potentiel et prometteur, susceptible d'avoir une incidence sur la croissance et la performance reproductive de T. swinderianus en captivité.

Mots-clés : Thryonomys swinderianus, croissance animale, Moringa oleifera, reproductif, régimes expérimentaux

Introduction

Livestock or wild animal domestication for meat production has been identified as conventional and non- conventional source of protein that could help substantially to bridge the gap between high demand for protein and the present low level of meat production (Adu *et al.,* 2010). Looking at the patterns of animal production in Nigeria, it's paramount that improvement is necessary and indeed desirable for variety of indigenous wild species such grass cutter, snailery, guinea fowl and queala birds. Thryonomys swinderianus is one of two species of grass cutters, a small family of African hystricognath rodents (Hoffmann, 2008; Matthews, 2008). The grass cutter lives by reed-beds and riverbanks in Sub-Saharan Africa. Grass cutter can grow to nearly 2 ft (0.61 m) in length and weigh a little less than 19 lb (8.6 kg). It has rounded ears, a short nose and coarse bristly hair. Its forefeet are smaller than its hind feet, each with three toes and are sometimes referred to as micro-livestock. Grass cutter lives in small groups led by a single male. They are nocturnal and make nests from grasses or burrow underground. Individuals of the species may live in excess of four years (Hoffmann, 2008; Matthews, 2008).

Grass cutter meat is very delicious and consumed by a lot of people in the West African sub-region (Adu *et al.,* 2010). Unfortunately, grass cutter production in captivity has been constrained by nutritional factors, which has made it lag behind other livestock with the result that a significant proportion of grass cutter meat consumed in the country have had to be hunted from the wild. Grass cutters are herbivorous, their favourite food being savannah grasses (Matthews, 2008). In captivity they also take sugar cane, corn stalks and cassava peeling. Therefore, uses of functional feed are novel in livestock industry, there are a large number of feed supplement available to improve livestock performance. World Health Organisation encourages using medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to the nature. Attempts to use the natural material such as Moringa oleifera (a medicinal plant) could be widely accepted as feed supplements to enhance efficiency of feed utilization and animal productive performance (Mohamed *et al.,* 2003).

Moringa oleifera belongs to family moringaceae. Moringa oleifera is native to India and introduced into the tropics (Mabruk *et al.,* 2010). It is a multipurpose tree that is grown in semi-arid and tropical areas. It is mainly used for human nutrition and for correction of malnutrition of kids in India and Southern America. The description of Moringa tree as a miracle tree coincides with its high nutritive value as human food beside its different medicinal uses.

Recent studies have shown successful use of M. oleifera in broilers chicken, rabbits and cow, pig and sheep nutrition (Gadzirayi *et al.,* 2012; Portugaliza and Fernandez, 2012; Bouatene *et al.,* 2011; Odeyinka *et al.,* 2008; Bryan *et al.,* 2007) respectively. At present the scientists are working to improve feed efficiency and growth rate of livestock using useful herbs (Bunyapraphatsara, 2007). Moringa oleifera can be used as growth promoter and reproductive enhancement but the mechanism

of action M. oleifera as a growth promoter and reproductive enhancer is yet to be adequately researched in livestock such as grass cutter. Hence, this present study was therefore to investigate the effect of M. oleifera on the growth, reproductive performance and survival of T. Swinderianus.

Materials and Methods

Plant Collection and Preparation

Moringa oleifera leaves were collected from the Nursery and Wood Processing Units of Department of Forest Resources Management, University of Ibadan, Nigeria. The plants were washed with distilled water and allowed to air dry at room/ambient temperature (25°C) for two weeks. The leaves were blended into fine powder and stored in air tight container (25°C) until required.

Experimental System

The experiment was carried out in ten one-tier cage (1.40x0.60x0.90 m) for 40 weeks in the Domestication Centre, Department of Wildlife and Ecotourism Management, University of Ibadan, Nigeria. Grass cutters (20) were acclimatized for three weeks before the experiment and judged to be of good general health based on complete physical examination which includes general body condition, the eyes, ear and body examination before the commencement of the experiment.

Experimental Procedure and Feeding Trials

Each treatment have two replicate, 2 T. swinderianus (1 male and female) of mean weight ranged 1125.50±3.75-1410.25±14.50 g were distributed into the cage. The experiment lasted 40 weeks during which the grass cutter were fed to satiation/ad libitum twice daily (8.00-9.00 am and 4.00-5.00 pm) in the morning and evening respectively. Measurement of the weight changes was performed monthly.

Diets Formulation

Other feed ingredients were bought, ground and mixed together to formulate 22% crude protein diet. Each diet mixture was treated separately and extruded through a 1/8 mm die mincer of Hobart A-200T pelleting machine (Hobart GmbH, Rhen-Bosch, Offenbug, Germany) to form noodle like strands, which were mechanically broken into suitable sizes for the grass cutters. The pellets were sun dried, packed in labelled polythene bags and stored in a cool dry place to prevent fungal growth (Table 1).

Biological Evaluation

Weight gain = final body weight - initial body weight

Weight gain (%) = 100 (final body weight - initial body weight)/ Initial body weight

Specific growth rate (SGR) = (Log Wf – Log Wi) x 100 / t (days)

Where, Log Wf = logarithm of the grass cutter final weight gain.

Log Wi = logarithm of the grass cutter initial weight, t = experimental period in days

Feed conversion ratio (FCR) = Feed intake (g) / Weight gain (g)

Protein efficiency ratio (PER) = Wet body weight gain (g)/ Crude protein fed

Survival rate (%) =

=initial number of grass cutter stocked – mortality / initial number of grass cutter stocked × 100

Protein intake = feed intake × percentage protein in diet /100

Analytical Methods

Experimental diets and Moringa oleifera were analyzed for their proximate composition according to the methods of Association of Official Analytical Chemists (AOAC, 2005).

Statistical Analysis

Proximate composition of the experimental diets, Growth and reproductive performance resulting from the experiment were subjected to one-way Analysis Of Variance (ANOVA) using Statistical Package for Social

Sciences 2006 version 15.0 (SPSS). Duncan multiple range test was used to compare differences among individual means.

Results

Nutrient composition of M. oleifera leaf

Nutrient composition (crude protein, crude fibre, crude fat, ash content and nitrogen free extract) of M. oleifera leaf was determined and the result is shown in table 2.

Proximate composition of experimental diets fed T. swinderianus

Proximate composition (crude protein, crude fibre, crude fat, ash content and nitrogen free extract) was analyzed and highest crude protein recorded in diet 3 and the lowest in diet 5. This result is presented in table 3.

Growth performance and nutrient utilization of T. swinderianus fed different graded levels of M. oleifera leaf

The growth performance and feed utilization in terms of body weight gain, feed conversion ratio, protein efficiency ratio, protein intake and specific growth rate was presented in Table 4.

Birth characteristics of T. swinderianus fed graded levels of M. oleifera

Birth characteristics (litter size, mortality, survival rate and percentage survival) were determined and the result was presented in table 5.

Birth weight of T. swinderianus fed graded levels of M. oleifera leaf

The mean weight of fawn, T. swinderianus and gestation week were presented in table 6.

Discussion

The leaves of M. oleifera are a good source of protein, fibre and minerals, elements that are vital for the growth and the health of animals (aquatic and terrestrial). The values obtained from the chemical analysis of Moringa leaves shows that the Dry Matter (DM) value of the Moringa leaves in this study was higher (88.62%) than the value reported by Mutayoba et al. (2011), who reported DM values of 87.20%. Also, this value was lower to the value reported by (Olugbemi *et al.,* 2010; Ogbe and John (2011). They reported the values of 93.7% and 96.79 respectively. However, the crude protein value reported by (Olugbemi *et al.,* 2010; Mutayoba et al. (2011) were higher (27.44%, 30.65%) respectively than the value obtained in this study (26.62%) and the value obtained by (Ogbe and John, 2011; Bouatene *et al.,* 2011) were lower (17.01%, 23.63%) respectively to the value obtained in this present study. The crude fibre, fat and ash contents reported by (Ogbe and John, 2011; Bouatene *et al.,* 2011) were lower than the values obtained in this study. These variations can be explained by differences in agro-climatic conditions, age of trees, genotype, environmental factors, post-harvest treatments, the season of harvesting and maturation stage of the leaves has a strong influence on the nutrient content of Moringa leaves

The diets used in the study were formulated to provide 22% crude protein for T. swinderianus, experimental diets were formulated with different levels of M. oleifera for T. swinderianus. The proximate composition of the diets showed highest moisture content in diet 4 (75% MO) and the lowest in control, the highest value of crude protein was recorded in diet 3 (50% MO) and lowest in diet 5 (100% MO) and there were not significant difference ($p>0.05$) among the treatments. The values of crude protein recorded in all treatments of this study were similar to the report by (McDonald *et al.,* 1995) who put the protein requirements for livestock raised in the tropics at 20-22% crude protein.

The effect of feed on the performance of T. swinderianus indicates that at the end of the experiment there was general increase in weight. The T. swinderianus showed good appetite for all the diet treatments attested to by the increase in body weight. The highest growth performance was observed in T. swinderianus on 100% M. oleifera (255.35±3.12 g).

The treated groups had a better growth than the control diet. There were significant differences ($p<0.05$) in the final body weight among the T. swinderianus fed on diet containing M. oleifera leaf-based diets and the

Table 1: Gross composition of experimental diets (g/100g).

Ingredients	Control (0% MO)	Diet 2 (25% MO)	Diet 3 (50% MO)	Diet 4 (75% MO)	Diet 5 (100% MO)
Soybeans	30.00	23.25	16.50	9.75	3.00
Palm kernel cake	10.00	16.75	23.50	30.25	37.00
Groundnut cake	35.00	26.25	17.50	8.75	-
Moringa oleifera leaf	-	8.75	17.50	26.25	35.00
Yellow maize	10.00	10.00	10.00	10.00	10.00
Wheat offal	7.50	7.50	7.50	7.50	7.50
Di-Calcium phosphate	2.50	2.50	2.50	2.50	2.50
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Salt	2.00	2.00	2.00	2.00	2.00
Vit–min premix *	2.00	2.00	2.00	2.00	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Key: MO= Moringa oleifera
** vit-min premix (vitamin and minerals premix) each 2.5kg of premix contains; vitamin A, 12.5 million international unit (MIU); D3,2.5 MIU; E, 40g; K3, 2g; B1, 3g; B2, 5.5g; B6, 5g; B12, 0.25g; Niacin 55g; Calcium pantothenate 11.5g; Choline chloride, 500g; folic acid, 1g; Biolin, 0.08g; Manganese, 120g; Iron, 100g; Zinc, 80g; Copper, 8.5g ; Iodine, 1.5g ; Cobalt, 0.3g ; Selenium, 0.12g ; Anti- oxidant, 120g.*

Table 2: Nutrient composition of M. oleifera harvested from Nursery and Wood Processing Units, Department of Forestry Resources Management, University of Ibadan.

Nutrients analyzed (% DW)	Mean composition (% ± SD)
Moisture	11.38±0.01
Dry matter	88.62±0.05
crude protein	26.62±0.02
crude fibre	18.97±0.07
Crude fat	5.34±0.06
Ash	12.01±0.10
NFE	25.68±0.03

Key: Data are mean values ± standard deviation (SD) of duplicate results; DW = dry weight.

Table 3: Proximate composition of experimental diets (DM)

	Control (0% MO)	Diet 2 (25% MO)	Diet 3 (50% MO)	Diet 4 (75% MO)	Diet 5 (100% MO)
Moisture	6.80±0.02^{a}	6.98±0.02^{c}	7.55±0.01^{d}	7.78±0.01^{e}	6.90±0.03^{b}
Crude protein	22.08±0.24^{a}	22.08±0.27^{a}	22.10±0.11^{a}	22.08±0.09^{a}	22.04±0.18^{a}
Ether extract	15.38±0.10^{c}	16.62±0.01^{e}	16.40±0.06^{d}	15.10±0.02^{b}	14.92±0.00^{a}
Ash	14.80±0.02^{a}	15.10±0.03^{b}	15.40±0.01^{c}	16.10±0.50^{e}	15.98±0.04^{d}
NFE	40.94±0.17^{e}	39.22±0.18^{c}	38.55±0.10^{a}	38.94±0.32^{b}	40.16±0.13^{d}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)
MO=Moringa oleifera

Table 4: Growth performances of T. swinderianus fed the experimental diets for 40 weeks.

Parameters	Control (0% MO)	Diet 2 (25% MO)	Diet 3 (50% MO)	Diet 4 (75% MO)	Diet 5 (100% MO)
Initial body weight(g)	1125.50±3.75^{a}	1380.15±5.00^{d}	1245.75±6.25^{b}	1290.20±7.50^{c}	1410.25±14.50^{e}
Final body weight (g)	1211.12±1.43^{a}	1505.57±2.50^{c}	1449.30±3.13^{b}	1518.01±3.72^{d}	1665.60±7.25^{e}
Body weight gain (g)	85.62±0.71^{a}	125.42±1.70^{b}	203.55±1.56^{c}	227.81±1.80^{d}	255.35±3.12^{e}
Body weight gain (%)	7.61±0.02^{a}	9.09±0.05^{b}	16.34±0.01^{c}	17.66±0.01^{d}	18.10±0.02^{d}
Daily weight gain (g)	0.28±0.00^{a}	0.40±0.01^{a}	0.61±0.02^{b}	0.75±0.00^{c}	0.88±0.01^{c}
Specific growth rate	0.01± 0.00^{a}	0.01± 0.00^{a}	0.02± 0.00^{a}	0.03± 0.00^{a}	0.03± 0.00^{a}
Protein efficiency ratio	3.88±0.02^{a}	5.68±0.01^{b}	9.21±0.02^{c}	10.32±0.01^{d}	11.59±0.00^{e}
Protein intake (g)	208.21±0.03^{a}	204.64±0.01^{a}	227.43±0.05^{c}	217.24±0.02^{b}	236.88±0.03^{d}
Food conversion ratio	0.11±0.00^{d}	0.07±0.01^{c}	0.05±0.02^{b}	0.04±0.00^{a}	0.04±0.00^{a}

Key: Mean followed by the same letter is not significantly different (p > 0.05) MO=Moringa oleifera

Table 5: Birth characteristics of T. swinderianus fed graded levels of Moringa oleifera

	Control (0% MO)	Diet 2 (25% MO)	Diet 3 (50% MO)	Diet 4 (75% MO)	Diet 5 (100% MO)
Litter size	5^{a}	4^{a}	4^{a}	5^{a}	5^{a}
Mortality	3^{b}	2^{ab}	1^{ab}	0^{a}	0^{a}
Survival Rate	2^{a}	2^{a}	3^{a}	5^{a}	5^{a}
Percentage Survival	40^{a}	50^{b}	75^{c}	100^{d}	100^{d}

Key: Mean followed by the same letter is not significantly different (p > 0.05) MO=Moringa oleifera

Table 6: Birth weight of T. swinderianus fed graded levels of M. oleifera leaf

	Control (0% MO)	Diet 2 (25% MO)	Diet 3 (50% MO)	Diet 4 (75% MO)	Diet 5 (100% MO)
Gestation week	34	36	37	32	32
Mean weight of fawn at birth (g)	131.52^{a}	131.00^{a}	130.06^{a}	131.85^{a}	135.00^{a}

Key: Mean followed by the same letter is not significantly different (p > 0.05) MO=Moringa oleifera

control. The result of this present study was in agreement with the report of (Gadzirayi *et al.,* 2012; Bouatene *et al.,* 2011; Portugaliza and Fernandez, 2012; Adeniji and Lawal, 2012) who reported better weight gain in Moringa oleifera leaf meal supplemented diets compared to the control. This result was in agreement with the findings of (Bamikole *et al.,* 2005) who reported an increase in weight gain after feeding rabbits with mulberry leaves based diet.

Feed Conversion Ratio (FCR) is used to assess feed utilization and absorption (conversion of feed to flesh). FCR was best (0.04±0.00) with diet 5 (100% MO) and least recorded in control (0.11±0.00), there were significant differences (p<0.05) among

the treatments. The result revealed that diet containing Moringa oleifera leaf at 100% inclusion was better utilized by T. swinderianus than the control diets. This present study support the report of (Portugaliza and Fernandez, 2012; Adeniji and Lawal, 2012) who reported better food conversion ratio in Moringa oleifera leaf meal supplement diets compared to control diets.

The result of the study also, showed that diet 5 (100% MO) recorded the highest value of protein efficiency ratio (11.59±0.00) and the lowest was recorded in the control diet (3.88±0.02), this result shows significant difference ($p<0.05$) among the treatments. Protein Efficiency Ratio (PER) is a measurement of protein effectiveness to provide the essential amino acids needed by the animals as well as an index that had been associated with fat deposition in animals muscles (DeSilva and Anderson, 1995). Feed Efficiency Ratio (FER) and Protein Efficiency Ratio (PER) are used as quality indicators for livestock diet and amino acid balance. So, these parameters are used to assess protein utilization and turnover.

The results of specific growth rate revealed that 75% and 100% MO (0.03±0.00g) had better growth rate compared to the control, although there were no significant differences among the treatments ($p>0.05$). This observation was similar to Azza and Abd-El-Rhman (2009) who found that the specific growth rate was not significantly affected by the dietary intake of propolis at 0.1 and 10 g propolis kg^{1} diet.

The performance of the Moringa supplemented feed could be explained by the presence of Moringa oleifera in the feed. Moringa has strengthened the content of the feed protein and fibre. Proteins of Moringa leaf have very high biological values (Bouatene *et al.,* 2011). All essential amino acids present in Moringa leaves are in a concentration greater than the minimum recommended by FAO and WHO mentioned in the feed reference that is groundnut cake (Bouatene *et al.,* 2011). These might attributed to the best performance of Moringa oleifera supplemented based diets.

The highest litter size was obtained in the control diet, diet 4 and 5 while the lowest were recorded in diet 2 and 3, they were no significant difference ($p>0.05$) among the treatments. This result is in accord with the report of (Ozimba and Lukefahr, 1991; Mai, 2005). Also, the result is similar to the report of (Odeyinka *et al.,* 2008) who recorded highest litter size in control diets and 100% inclusion of Moringa oleifera supplemented diets.

At the end of the experiment highest mortality was observed in the control diet (3) while the lowest was recorded in diet 4 and 5 (with no mortality) and these shows significant difference ($p<0.05$) among the treatments. The best percentage survival was recorded in diet 4 (75% MO) and 5 (100% MO) while the least in the control diet (40%). There were significant differences ($p<0.05$) among the treatments. This result contradicts the report of (Odeyinka *et al.,* 2008) who recorded highest percentage survival in the control diets.

The highest mean weight of fawn, T. swinderianus at birth was recorded in the diet 5 (100% MO) 135.00 g on the 32 weeks and closely followed by diet 4 (131.85 g) in 32 weeks, although there were significant differences ($p<0.05$) in the birth weight of the fawn on diet 4 and 5 while the lowest mean birth weight were recorded diet 2 and control diet (131.00 g, 131.52 g) in 36 and 34 weeks respectively. They were no significant difference ($p<0.05$) between diet 2 and control diet. This result support the report of (Odeyinka *et al.,* 2008) who obtained highest litter weight at birth in rabbit fed 100% inclusion of M. oleifera supplemented diets.

Thus, M. oleifera supplemented based diets shows best performance compared to control. This could be attributed to complete amino acids, considerable amount of vitamins, minerals, antioxidants, immunostimulants and antibacterial compounds such as pterygospermin (Anwar *et al.,* 2007; Fahey, 2005; Makkar and Becker, 1997). The complete nutritional components and some growth stimulating compounds of M. oleifera probably compensated for the enhancement of the live weight and reproductive performance. In addition, the few amount of anti-nutritional factors that affect palatability of feeds (Kakengi *et al.,* 2007) were not implicated to compromise

the bioavailability of nutrients and growth stimulating compounds present in M. oleifera leaves (Foidl *et al.,* 2001).

Conclusion

The supplementation of M. oleifera leaf meal at 100% in the feed to replace ground nut cake gives the best results in terms of gross weight, growth rate, feed utilization/efficiency and survival of young T. swinderianus (fawn). The performances have been achieved due to the high digestibility of its proteins and its antimicrobial activity. Moringa oleifera leaf could be useful as feed supplement and as medicine in grass cutter production to improve health (reproduction) and growth performance.

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References

Adeniji, A.A. and M. Lawal, 2012. Effects of replacing groundnut cake with moringa oleifera leaf meal in the diets of grower rabbits. Int. J. Molecular Vet. Res., 2: 8-13. DOI: 10.5376/ ijmvr.2012. 02.0003

Adu, E.K, H. Bagulo and K. Amaning-Kwarteng, 2010. The effect of incorporation of Moringa oleifera leaves and sweet potato vines in the ration of growing grass cutters (Thryonomys swinderianus Temminck). Livestock Res. Rural Develop., 22: 1-8.

Anwar, F.S., M.A. Latif and H. Gilani, 2007. Moringa oleifera: A food plant with multiple medicinal uses. Phytotherapy Res., 21: 7-25. PMID: 17089328

A.O.A.C. 2005. Official methods of analysis. Association of Official Analytical Chemists, Washington, DC, 18th edition

Azza, M. and M. Abd-El-Rhman, 2009. Antagonism of Aeromonas hydrophila by propolis and its effect on the performance of Nile tilapia, Oreochromis niloticus. Fish Shellfish Immunol., 27: 454-459. DOI: 10.1016/j.fsi.2009.06.015

Bamikole, M.A., M.I. Ikhatua, U.J. Ikhatua and I.V. Ezenwa, 2005. Nutritive value of mulberry (Morus spp.) leaves in growing rabbits in Nigeria. Pak. J. Nutrition, 4: 231-236. http://www.docsdrive.com/pdfs/ansinet/pjn/2005/231-236.pdf

Bouatene, D., L.G. Bohoua and S. Dabonne, 2011. Effect of Moringa oleifera on growth performance and health status of young post-weaning rabbits. Res. J. Poultry Sci., 4: 7-13. DOI: 10.3923/rjpscience.2011.7.13

Bryan, M., R. Nadir and R. Rosario, 2007. Successful experiences on the use of Moringa oleifera in animal feeding.

Bunyapraphatsara, N., 2007. Utilization of medicinal plants in animal production. Chickens Ann. Anim. Sci., 10: 157-165.

DeSilva, S.S. and T.A. Anderson, 1995. Fish Nutrition in Aquaculture. Chapman and Hall Aquaculture Series, Tokyo.

Fahey, J.W., 2005. Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Trees Life J., 1: 5-5.

Foidl, N., H.P.S. Makkar and K. Becker, 2001. The potential of moringa oleifera for agricultural and industrial uses. In: The Miracle Tree/The Multiple Attributes of Moringa oleifera. Fuglie, L.J., (Ed.). CTA, USA.

Gadzirayi, C.T., B. Masamha, J.F. Mupangwa and S. Washaya, 2012. Performance of broiler chickens fed on mature Moringa oleifera leaf meal as a protein supplement to soyabean meal. Int. J. Poultry Sci., 11: 5-10.

Hoffmann, M., 2008. Thryonomys swinderianus. In: IUCN 2008. IUCN Red List of Threatened Species.

Kakengi, A.M.V., J.T. Kaijage, S.V. Sarwatt, S.K. Mutayoba and M.N. Shem *et al.,* 2007. Effect of moringa oleifera leaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. Livestock Res. Rural Develop., 19: 120-120.

Mabruk, A. A, Talib, H. N, Mohamed, M. A, Alawad, A. H. 2010. A note on the potential use of moringa oleifera tree as animal feed. U of K. J. Vet. Med. and Anim. Prod. Vol. 1 (2): 184-188

Mai, H.T.X., 2005. Rabbit feeding and Management. Agricultural Publishing House, Ho Chi Minh City, Vietnam.

Makkar, H.P.S. and K. Becker, 1997. Nutrients and anti-quality factors in different morphological parts of the Moringa oleifera Tree. J. Agric. Sci., 128: 311-322.

Matthews, J., 2008. The Value of Grasscutters. World Ark, (January-February, 2008), pp. 23-24.

McDonald, P., R.A. Edwards, J.F.O. Greenhalgh and C.A. Morgan, 1995. Animal Nutrition. 4th Edn., John Wiley and sons, United State, pp: 607.

Mohamed, A.H., B.E. El-Saidy and I.A. El-Seidy, 2003. Influence of some medicinal plants supplementation: 1-On digestibility, nutritive value, rumen fermentation and some blood biochemical parameters in sheep. Egyptian J. Nutrition Feeds, 6:139-150.

Mutayoba, S.K., E. Dierenfeld, V.A. Mercedes, Y. Frances and C.D. Knight, 2011. Determination of chemical composition and anti-nutritive components for Tanzanian locally available poultry feed ingredients. Int. J. Poultry Sci., 10: 350-357.

Odeyinka, S.M., O.J. Oyedele, T.O. Adeleke and J.A. Odedire, 2008. Reproductive performance of rabbits fed Moringa oleifera as a replacement for Centrosema pubescens. 9th World Rabbit Congress, Reproduction, June 10-13, Verona, Italy. 411- 416

Ogbe, A.O. and P.A. John, 2012. Proximate study, mineral and anti-nutrient composition of Moringa oleifera leaves harvested from Lafia, Nigeria: Potential benefits in poultry nutrition and health. J. Microbiol. Biotechnol. Food Sci., 1: 296-308.

Olugbemi, T.S., S.K. Mutayoba and F.P. Lekule, 2010. Effect of Moringa (Moringa oleifera) inclusion in cassava based diets to broiler chickens. Int. J. Poultry Sci., 9: 363-367.

Ozimba, C.E. and S.D. Lukefahr, 1991. Comparison of rabbit breed type of post weaning litter, growth, feed efficiency and survival performance traits. J. Anim. Sci., 69: 3494-3500.

Portugaliza, H.P. and T.J. Fernandez, 2012. Growth performance of Cobb broilers given varying concentrations of malunggay (Moringa oleifera Lam.) aqueous leaf extract. Online J. Anim. Feed Res., 2: 465-469.

PERFORMANCE CHARACTERISTICS OF WEANER RABBITS FED MORINGA OLEIFERA AND MORINGA STENOPETALA

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Abstract

This study was designed to investigate the utilization of Moringa oleifera (MO) and Moringa stenopetala (MS) by weaner rabbit. In a twelve week feeding trial, forty eight weaner rabbits of about five weeks old were allotted into three treatments with each treatment consisting of sixteen rabbits in a completely randomized design. Animals in T1 were fed 50% basal diet and 50% MO, T2 50% basal diet, 25% MO and 25% MS and T3 50% basal diet and 50% MS respectively. They were fed at 4% of their body weight. Parameters measured included; feed intake, weight gain, carcass and haematological characteristics. At the end of the experiment, five rabbits from each treatment were selected based on similarity in weight and were slaughtered for carcass and haematological evaluations. The proximate analysis of the experimental diets was carried out according to the standard procedure of AOAC (2000). The data obtained were statistically analyzed with the General Linear Model of SAS (2008) and the Duncan New Multiple Range Test option of SAS (2008) was used to detect significant differences among means. The daily weight gain of T1 (8.11) and T2 (7.64) were significantly higher ($p<0.05$) than T3. There was significant difference ($p<0.05$) in the digestibility coefficient of the animal across the treatments. It can be concluded from the study that feeding Moringa oleifera and Moringa stenopetala does not have deleterious effect on the performance and carcass qualities of weaner rabbits.

Keywords: Performance, Moringa oleifera, Moringa stenopetala

CARACTÉRISTIQUES DE PERFORMANCE DES LAPINS SEVRÉS NOURRIS AVEC DES FEUILLES DE MORINGA OLEIFERA ET MORINGA STENOPETALA

Résumé

La présente étude a été conçue dans le but d'étudier l'utilisation de feuilles Moringa oleifera (MO) et Moringa stenopetala (MS) dans l'alimentation des lapins sevrés. Dans un essai alimentaire de douze semaines, quarante-huit lapins sevrés, âgés d'environ cinq semaines, ont été affectés à trois traitements, chaque traitement étant composé de seize lapins dans un schéma complètement aléatoire. Les animaux au T1 ont été nourris avec un régime dans les proportions de 50% régime de base et 50% MO ; le T2 comportant 50% régime de base, 25% MO et 25% MS ; et le T3 composé de 50% régime de base et 50% MS. Les lapins ont été nourris à 4% de leur poids corporel. Les paramètres mesurés comprenaient la prise alimentaire, le gain pondéral, les caractéristiques de carcasse et les caractéristiques hématologiques. A la fin de l'expérience, cinq lapins de chaque traitement ont été sélectionnés sur la base de la similitude du poids de leurs carcasses et ont été abattus pour des évaluations de la carcasse et des paramètres hématologiques. L'analyse quantitative de la composition des régimes expérimentaux a été réalisée selon la procédure standard de l'AOAC (2000). Les données obtenues ont été analysées statistiquement en utilisant le modèle linéaire général de SAS (2008), et le nouveau test de comparaisons multiples de Duncan (Duncan New Multiple Range Test Option of SAS - 2008) a été utilisé pour détecter des différences significatives entre les moyennes. Le gain pondéral moyen quotidien du T1 (8.11) et du T2 (7,64) était significativement plus élevé ($p <0,05$) que celui du T3. On a noté une différence significative ($p <0,05$) au niveau du coefficient de digestibilité chez les animaux à travers les traitements. On peut conclure de l'étude que l'alimentation comportant des feuilles de Moringa oleifera et Moringa stenopetala n'a pas d'effet néfaste sur la performance et les qualités de la carcasse de lapins sevrés.

Mots-clés : performance, Moringa oleifera, Moringa stenopetala

Introduction

Tropical forage of good quality is available for limited period of the year (during and shortly after rainy season). Moringa is resistant to drought and this could serve as alternative feedstuff in animals' diet during the dry season. Rabbit production is encouraged in Nigeria as a means of improving the daily protein intake of individuals (Ekpenyong and Biobaku, 1986) especially because of its short gestation period. Rabbit production is promising in Nigeria but grains are expensive and scarce but forages are cheap and abundant. The use of Moringa oleifera as feed for rabbits is limited and Moringa stenopetala has not been fed to rabbits in Nigeria. This study was therefore designed to investigate the performance characteristics of weaner rabbits fed Moringa oleifera (MO) and Moringa stenopetala (MS).

Material and Methods

The experiment was carried out at the Rabbit Unit of Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Osun State, Nigeria. In a twelve week feeding trial, forty eight weaner rabbits of about five weeks old were allotted into three treatments with each treatment consisting of sixteen rabbits in a completely randomized design. Animals in T1 were fed 50% basal diet (Table 1) and 50% MO, T2 50% basal diet, 25% MO and 25% MS and T3 50% basal diet and 50% MS respectively. They were fed at 4% of their body weight. Parameters measured included; feed intake, weight gain and apparent digestibility coefficient. The proximate analysis of the experimental diets was carried according to the standard procedure of AOAC (2000). The data obtained were statistically analyzed with the General Linear Model of SAS (2008) and the Duncan New Multiple Range Test option of SAS (2008) was used to detect significant differences among means.

Table 1: Composition of the basal diet

Ingredient	Percentage (%)
Palm kernel cake (PKC)	50.00
Wheat offal	23.00
Maize	15.00
Groundnut Cake (GNC)	8.00
Bone meal	2.00
Fish meal	1.00
Lysine	0.25
Methionine	0.25
Vitamin premix	0.25
Salt	0.25

Results

Table 2: Chemical composition of the basal diet and forages

Parameter	Concentrate	Moringa oleifera	Moringa stenopetala
Dry matter	92.64	14.61	18.48
Analysis % of DM			
Crude protein	17.20	22.45	27.20
Crude fibre	7.36	6.75	14.63
Ether extract	7.50	8.02	8.72
Ash	9.06	7.99	10.94
Nitrogen free extract	51.41	46.98	30.31
Organic matter	90.94	92.01	89.06

Table 3: Apparent digestibility coefficient of the dry matter and nutrients of experimental rabbits

Parameter (%)	T1	T2	T3	SEM
Digestible Dry matter	86.86[a]	86.74[a]	77.56[b]	11.86
Digestible Crude protein	90.61[a]	88.23[a]	87.73[a]	2.25
Digestible Ether extract	92.57[a]	92.60[a]	89.68[b]	0.89
Digestible Crude fibre	64.91[b]	72.00[a]	57.62[c]	1.17
Digestible Ash	34.95[a]	35.93[a]	41.13[a]	0.80
Digestible Nitrogen free extract	93.84[a]	48.30[c]	64.84[b]	7.48

Mean within each row with different superscript are significantly different (p<0.05)

Note; T1: 50% basal diet and 50% Moringa oleifera
T2: 50% basal diet, 25% Moringa oleifera and 25% Moringa oleifera
T3: 50% basal diet and 50% Moringa stenopelata
SEM: Standard error of mean

Table 4: Performance characteristics of weaner rabbits fed Moringa species Forages

Parameter	T1	T2	T3	SEM
Dry matter intake (g/day)	37.90[a]	31.91[a]	25.58[b]	1.29
Initial weight (g)	475.63[a]	471.25[a]	474.38[a]	27.48
Final weight (g)	1,156.88[a]	1,113.13[a]	893.13[b]	32.76
Total weight gain (g)	681.25[a]	641.88[a]	418.75[b]	27.40
Daily weight gain (g/day)	8.11[a]	7.64[a]	4.99[b]	0.33

Mean within each row with different superscript are significantly different (p<0.05)

Note; T1: 50% basal diet and 50% Moringa oleifera
T2: 50% basal diet, 25% Moringa oleifera and 25% Moringa oleifera
T3: 50% basal diet and 50% Moringa stenopelata
SEM: Standard error of mean

Discussion

Table 2 shows the chemical composition of the basal diet and forages. It shows that the dry matter, crude protein, crude fibre, ash content of Moringa stenopetala was higher than that of Moringa oleifera while having lower nitrogen free extract and organic matter content. Table 3 shows the apparent digestibility coefficient of the experimental rabbits. There was significant difference (p<0.05) in the digestibility coefficient of the animal across the treatments. Rabbits fed T1 and T2 diets had significantly higher dry matter and crude protein digestibility coefficient than T3. This agreed with the findings of Sanchez *et al.,* (2005) who reported that the inclusion of Moringa oleifera as a protein supplement to low quality diet improved dry matter digestibility of the diet.

Table 4 shows the performance characteristics of weaner rabbits fed Moringa spp. Rabbits fed 50% basal diet and 50% Moringa stenopetala (T3) had the least performance characteristic. The mean dry matter feed intakes were 37.90, 31.91 and 25.58g for rabbits fed T1, T2 and T3 diets respectively. There was significant difference (p<0.05) between the mean dry matter intakes. The mean dry matter intake of rabbits fed T3 diet was significantly (P<0.05) lower than that of others. The dry matter intake in this study was lower than 48.40 – 49.60g/day reported by Odeyinka *et al.,* (2007).

The mean daily weight gains (g/day) were 8.11, 7.64 and 4.99g/day/animal for rabbits fed T1, T2 and T3 diets respectively. There was significant difference (p<0.05) between the mean daily weight gains. The mean daily weight gain of rabbits fed T3 diet was significantly (P<0.05) lower than that of

others. The inclusion of Moringa species in the diet of rabbits brought about increased palatability and daily feed intake.

Conclusion

It could be concluded from the study that feeding Moringa oleifera and Moringa stenopetala does not have deleterious effect on the performance characteristics of weaner rabbits.

References

AOAC 2000. Association of official analytical chemists. Official methods of Analysis 16th edition. Washington D.C USA.

Ekpeyong T.E. and Biobaku W.O. 1986. Growth response of rabbits fed activated sewage and dried poultry waste. Journal of Applied Rabbit Research 1: 14-16.

Odeyinka, S.M., Olosunde, A.S. and Oyedele, O.J. 2008. Utilization of soybean milk residue, cowpea testa and corn starch residue by wearner rabbits. Livestock Research for Rural Development 19 (9) 2007.

SAS, 2008. SAS Users Guide Statistics, SAS inc. Cary, North Califonia, 2008 edition.

Sanchez, N.R., Spondly, E. and Ledin, I. 2005. Effect of feeding different levels of foliage Moringa oleifera to creole dairy cows on intake, digestibility, milk production and composition. http:/www.moringanews.org/documents/LivestProd.pdf. Accessed on 10/02/07.

GASTRO-INTESTINAL NEMATODES OF GOATS REARED UNDER COMMUNAL SMALL SCALE FARMING CONDITIONS IN BOTSWANA

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Abstract

The study was carried out in goats reared under communal small scale farming conditions near Gaborone, Botswana, to evaluate two sample preparation methods for McMaster egg counting technique, determine worm egg counts (WEC), identify nematode genera by larval culture, and determine abomasal and intestinal worm counts. Goat faecal samples (n = 30) were collected, split, and paired for preparation by either the simple method or the standard method and WEC was performed using the McMaster technique. The simple method resulted in significantly higher WEC than the standard method (p =0.04). The WEC of a cohort of goats from Modipane communal small scale farmers (n = 30) was found to be 995±229 eggs per gram (EPG). Larval culture of Modipane herds identified Trichostrongylus spp at 86 percent and Haemonchus spp, Ostertagia spp, Oesophagostomum spp, and Chabertia spp all occurring at less than 5 % each. Abomasal worm counts of two separate groups of goats at slaughter were found to be 110 ± 28 Haemonchus spp (n = 21) and 277 ± 129 Haemonchus spp (n = 30) in goats with WEC of 836 ± 236 EPG and 642 ± 120 EPG respectively. Intestinal worms were not found in the group (n = 30) that was tested for them. It was concluded that there is a significant worm burden among goats reared under communal small scale farming conditions in Botswana.

Key words: Nematodes, McMaster technique, goats, abomasal worm count, worm burden

NÉMATODES GASTRO-INTESTINAUX DES CHÈVRES ÉLEVÉES EN SYSTÈME COMMUNAUTAIRE ARTISANAL AU BOTSWANA

Résumé

L'étude a été réalisée sur des caprins élevés en système communautaire artisanal près de Gaborone (Botswana), dans le but d'évaluer deux méthodes de préparation d'échantillons pour la technique McMaster de comptage des œufs, de déterminer la numération des œufs de vers (WEC), d'identifier les genres de nématodes par la culture larvaire, et déterminer la numération des vers de caillette et intestinaux. Des échantillons d'excréments de chèvres (n = 30) ont été prélevés, divisés et jumelés pour la préparation soit par la méthode simple ou par la méthode standard, et la WEC a été réalisée en utilisant la technique McMaster. La méthode simple a révélé une WEC significativement plus élevée par rapport à la méthode standard (p = 0,04). La WEC d'une cohorte de chèvres des petites exploitations communautaires de Modipane (n = 30) a été de 995 ± 229 œufs par gramme (EPG). La culture larvaire dans les troupeaux de Modipane a identifié Trichostrongylus spp à 86 pour cent et Haemonchus spp, Ostertagia spp, Oesophagostomum spp, et Chabertia spp à un taux de moins de 5% chacun. Les nombres de vers de caillette de deux groupes distincts de chèvres à l'abattage ont été établis à 110 ± 28 Haemonchus spp (n = 21) et 277 ± 129 Haemonchus spp (n = 30) chez les chèvres avec respectivement une WEC de 836 ± 236 EPG et 642 ± 120 EPG. Les vers intestinaux n'ont pas été trouvés dans le groupe (n = 30) testé pour leur détection. Il a été conclu que les caprins élevés en système communautaire artisanal au Botswana ont une charge parasitaire importante.

Mots-clés : nématodes, technique McMaster, chèvres, nombre de vers de caillette, charge parasitaire

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Introduction

Infection with gastro-intestinal nematodes is common in domestic ruminants world-wide and has a significant economic impact particularly in the tropics and sub-tropics. While heavy nematode burdens can cause mortality, these parasites largely limit productivity (Sweeny *et al.,* 2012). The control of nematode infection in livestock is limited by the high cost of anthelmintics. Also, increasingly, the parasites develop anthelmintic resistance (Stafford *et al.,* 2009; Morgan and Coles, 2010). The nature of gastro-intestinal nematode infection in goats among small scale rural farming communities in Botswana is undocumented and hence unknown. Anecdotal evidence suggests these communities hardly control the parasites in their herds for various reasons including cost limitations and lack of awareness of the extent to which nematode infection limit productivity.

To address the needs of small scale goat farmers in rural areas it is necessary to carry out empirical studies to determine the nature of gastro-intestinal nematodes among livestock including worm burdens, determination of the prevalent genera and their faecal egg output (Ratanapob *et al.,* 2012). In the current study the nature of gastro-intestinal nematode infection in goats reared under small scale farming conditions was investigated. Detection and quantitation of nematode infection in goats is still widely dependent on the McMaster technique owing to the expense required for molecular diagnostic techniques (Rinaldi *et al.,* 2011; Vadlejch *et al.,* 2011). Firstly, a simple sample preparation method that requires less time and equipment and is increasingly being used in laboratories (http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e05.htm#TopOfPage), was evaluated against the standard method for preparing samples for McMacster worm egg counting technique. Secondly, consistent with the necessity to determine the level of nematode infection to inform intervention measures (Tsotetsi *et al.,* 2013), the worm burden among goats under communal small scale farming conditions was estimated using the selected faecal egg counting technique. Thirdly, larval culture was used to identify the nematode genera constituting the strongyle eggs in the goat faeces. The identity of nematode genera is important owing to the variation in the pathogenicity between different genera (van Wyk *et al.,* 2004; http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e05.htm#TopOfPage). Lastly, abomasal and intestinal worm counts were carried out on goats at slaughter to determine the nematode genera most common in goats from small scale rural communities. The empirical findings from this study are discussed in the context of the role of gastro-intestinal nematode in the productivity of small scale goat herds in rural communities.

Materials and Methods

Comparison of two sample preparation methods for McMaster worm egg counting technique. To compare two sample preparation methods for McMaster worm egg counting thirty (30) goats were randomly selected from a herd known to be infected with nematodes. Faecal material was collected from the rectum of each goat. Each sample was split into two equal amounts and paired so that one part was prepared using a simple method and the other sample prepared by the standard method. Briefly, in the simple method, a small bowl containing a sieve was placed on a balance and used to weigh two grams of faecal material. Twenty eight millilitres of saturated saline was added to the bowl and a spatula was used to homogenise the faecal matter. Any material remaining in the sieve was discarded. The homogenate was loaded into chambers of a McMaster slide for worm egg counting. The standard method was carried out as previously described (Nsoso *et al.,* 2000). Briefly, the faeces were crushed first with a mortar and pestle and then two grams was put in a bottle containing forty-five glass beads and 28 mL of tap water. The bottle was tightly closed and shaken and the contents strained through a sieve. The fluid was centrifuged for two minutes at 1500 revolutions per minute. The supernatant was removed and replaced volume per volume with saturated salt solution. The contents were

mixed with a wooden applicator followed by inverting the tube five times. The mixture was loaded into chambers of a McMaster slide for worm egg counting. For samples prepared by both methods, worm egg counting was carried out as previously described (Nsoso *et al.,* 2000). The mean (± standard error) was determined for each method and the two methods were compared using a paired t-test and statistical significance was determined at 5% level.

Determination of nematode egg counts among goats in small scale farming communities. We identified a community of subsistence farmers at Modipane village in Botswana (24 0 37' S 270 23'E). The farmers were rearing goats in a setup typical of most small scale farmers in Botswana. The goat population per kraal averaged 30 animals. The goats were indigenous breeds reared with little or no anthelmintic treatment. We randomly selected 30 adult goats of mixed sex from three kraals (ten goats per kraal) and collected faecal materials from the rectum of each goat. Using the simple sample preparation method described above, we carried out worm egg counts and determined the mean strongylate egg count for the area.

Identification of nematode genera. To identify the nematode genera represented in the strongylate eggs, larval culture was performed using faecal material from Modipane herds described above and larvae recovered were identified to genus level morphologically and enumerated. Faecal matermlal collected from goat recta were pooled together for culture. Cultures were achieved by homogenising a mixture of faeces and 20% vermiculite (weight per weight), moistened with distilled water in a honey jar bottle. The bottles were closed loosely and had a large air space at the top. Incubation was at 27 degrees Celsius for 7 days with periodic checking to ensure samples do not dry up.

The larvae were recovered using the Baermann technique as previously described (www.rvc.ac.uk). Briefly, a plastic funnel with a rubber tube was clamped on a stand. The tube was closed with a clip. Ten grams of cultured faeces in a gauze was put in a tea strainer and placed in the funnel. Distilled water was poured into the funnel until the faeces were submerged. The set up was left overnight. The first 15 mL run off was collected into a tube and centrifuged at 1500 rpm for 2 minutes to sediment the larvae. All but 0.5 mL of the supernatant was removed. An equal volume (0.5 mL) of 5% formalin was added to the larvae as a preservative and the sample was stored in a refrigerator.

To identify larvae, a drop of larvae suspension was placed on a slide. The larvae were stained with Lugol's iodine. Larvae were identified based on characteristic features including total length, oesophagus length, tail sheath length and the number of intestinal cells as previously described by different workers (Lyndal-Murphy, 1987; Van Wyk *et al.,* 2004; Indre *et al.,*2009; Van Wyk and Mayhew, 2013; www.rvc.ac.uk). Free-living larvae were recognised by absence of the sheath. A key described in Massey University MVS 16.602 Diagnostic Pathology study guide was used to place larvae in the correct genus. A micrometer was used to separate the short tailed larvae (< 40 µm); Trichostrongylus (less than 720 µm in total length) from Ostertagia (over 720 µm in total length). Haemonchus spp were identified on the basis of medium-tailed larvae (50 – 60 µm) and not having a muscular band across the anterior part of the oesophagus, a feature that is characteristic of Cooperia. Also Haemonchus larvae were identified by their tapered bullet-shaped heads. A100 larvae were counted and tallied into different genera.

Abomasal and small intestinal worm counts. Two groups of goats were subjected to worm counts. The goats were slaughter age or older and of mixed sex. They came from small scale communal farmers in the catchment areas of Gaborone city council abattoir (24039'S 25054'E). The first group (n =21) were subjected to abomasal worm count. The second group (n =30) were subjected to both abomasal and intestinal worm counts. The worm egg count for both groups was determined by the simple method described above. To carry out worm counts the entire gastrointestinal tract was removed from the carcass. Two string ligatures were tied around the small intestine not more than 2 cm distal

to the pylorus. To remove the abomasum, a cut was made between the 2 ligatures and a second cut made through the distal part of the omasum. The abomasum was placed in a bucket and opened along its length. A small flow of water was used to wash abomasal contents into a bucket. The mucosa was washed thoroughly to remove all material adhering to the folds. The bucket contents were made up to 2 litres. While mixing vigorously with a stick, a tenth (200 mL) of the bucket contents was ladled out into a beaker. The sample was passed through a sieve and a steady flow of water was used to wash the sieve clear. The sample was made up to 500 mL with water. While mixing, 50 mL amounts were removed into a smaller beaker. This sub-sample representing 1/100th of the original contents, was examined on a white tray. The sample was stained with Lugol's iodine for 3 minutes then decolourised with 5% sodium thiosulphate leaving the worms dark brown. A quick count and identification of the worms was done with a hand lens and a more detailed identification using a dissecting microscope. The worms were identified to genus level. To determine the total worm burden the results of the subsample was multiplied by 100.

For small intestinal worms, the first 10 m of the small intestine was stripped from the mesentery starting from the pylorus and cut off. Scissors were used to open this section of the small intestine along its length. Under a trickle of water the contents of the mucosa was squeezed with fingers into a bucket. The small intestinal contents were processed as described above for abomasal contents to determine the total worm count and identify nematode genera.

Results

Comparison of two sample preparation methods for McMaster faecal egg counting technique. The faecal worm egg count of goats infested with nematodes was found to be significantly higher ($p = 0.04$) when samples were prepared using the simple method (958.7±159 EPG) compared to the standard method (528.7±122 EPG) (Fig. 1).

Determination of nematode egg counts among goats in small scale farming communities. The mean worm egg count (n = 30) was found to be 995±229 EPG. All the goats were positive for strongylate eggs.

Identification of nematode genera. To determine the nematode genera infecting the goats we carried out larval culture and identification of larvae to genus level using physical and morphological features. Enumeration of the larvae revealed that Trichostrongylus spp was the most prevalent at 86% and that the other nematodes detected being Ostertagia spp, Haemonchus spp, Oesophagostomum spp, and Chabertia spp occurred at less than 5% each (Fig. 2).

Abomasal and small intestinal worm counts. Goats (n = 21) subjected to abomasal worm counts only, were found to have 110 ± 28 Haemonchus spp worms and a worm egg count of 836 ± 236 EPG. The individual prevalence of worms was 57 percent (12/21). All the goats were positive for strongylate eggs.

Goats tested for both abomasal and intestinal worms (n = 30) were found to have 277 ± 129 Haemonchus spp worms in the abomasum and no worms in the intestines. The worm egg count of this group was 642 ± 120 EPG. The individual prevalence of worms in this

Text box 1

The most common infection of goats is round worms. They significantly reduce productivity and may even cause deaths in a herd. There is need to develop tools to detect goats that have roundworms and such tools ought to be simple and inexpensive. This study establishes a technique that simplifies the detection of round worm eggs in faeces of goats. The technique is then used to determine worm burden in goats that are reared under communal small scale farming conditions in Botswana. The study also hatches eggs in the faeces to get larvae and then uses the characteristics of the larvae to identify the type of roundworms infecting the goats. The presence of wireworms and their quantity in the stomach of goats at the time of slaughter was also determined. The study highlights the need to establish worm burden in goats reared under traditional farming conditions. The findings can be used to inform intervention measures targeted at improving the productivity of goats among small scale farmers.

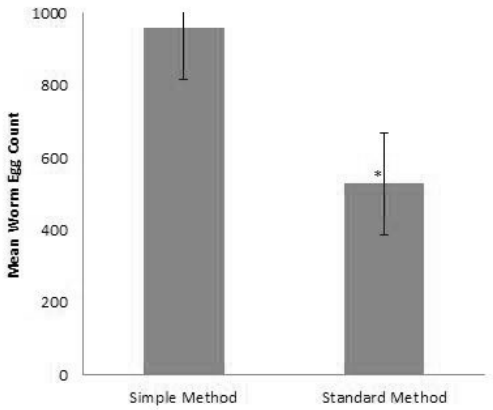

Figure 1: A comparison of two sample preparation methods for McMaster faecal egg counting technique. Goat faecal samples (n = 30) were split and paired samples were prepared by either the simple method or the standard method and faecal egg count was performed using the McMaster technique. Mean egg counts (mean ±SE) were calculated and compared using the paired t-test. * p = 0.04

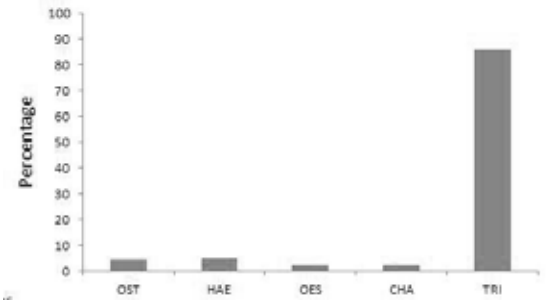

Figure 2: Percentage of larvae identified from cultured goat faeces. Pooled faecal material from nematode infected goats were cultured and larvae recovered by the modified Baermann funnel procedure. Larvae were microscopically examined and enumerated into different genera using their morphological features. OST: Ostertagia spp, HAE: Haemonchus spp, OES: Oesphagostomum spp, CHA: Chabertia spp and TRI; Trichostrongylus spp.

group was 43 percent (13/30).

Discussion

Based on the data from the current study we report that the simple method is more effective and yields higher worm egg counts than the standard sample preparation method for McMaster worm egg counting technique. The McMaster worm egg counting technique is the most common method used for the diagnosis of nematode infestation and to estimate worm burden in livestock including goats (Sweeny *et al.,* 2012). As previously described (Nsoso *et al.,* 2000), the standard sample preparation method for the technique requires a lot of materials that in the simple method are replaced by a sieve and a spoon. In addition, the simple method is quicker both advantages making this method attractive both as a diagnostic and a research tool. The findings of the current study suggest that the simple method is most suitable to use in less equipped laboratories such as in developing countries without compromising the accuracy of both research results and disease diagnosis. In deed even where equipment is abundant laboratories appear to prefer the simple sample preparation method over the standard one.

Decreased productivity of small ruminants due to infection with nematodes is very common across the world including the sub-tropical regions (Tsotetsi *et al.,* 2013). While it is widely recognised that goats reared under communal grazing areas are endemically infected with nematodes, there is a paucity of information on their worm burden as they are not regularly tested for worm egg counts. The current study determined the worm burden in goats under communal small scale farming conditions to be 995±229 eggs per gram. This finding is consistent with that of Totetsi *et al.,* (2013) who reported that most sheep and goats belonging to small scale farmers in South Africa had 100 – 1000 eggs per gram and most herds had a nematode prevalence above 50 percent. Ratanapop *et al.,* (2012) reported a slightly higher strongyle worm egg count (1176 EPG) among goats in Thailand. Worm burden above 1000 EPG in goats and sheep at herd level as compared to individual animal level are considered significant and should necessitate anthelmintic treatment. Segwagwe and Ramabu (1999) reported that helminthiasis and coccidiosis accounted for 43% and 40% of sheep and goat deaths respectfully in Botswana. Thus at flock level the worm burden reported here could be causing mortalities.

Nematodes cannot easily be identified at genus level by examination of eggs. In fact several genera are often lumped together as

strongyles when worm burden is expressed using faecal egg counts. Larval identification revealed that Trichostrongylus spp was the most prevalent nematode in contrast with the finding by Totetsi *et al.,* (2013) who reported Haemonchus spp to be more common than Trichostrongylus spp in sheep and goats. Interpretation of larval culture results as is that of faecal egg counts should consider variability resulting from culture conditions which may increase the hatchability of one species over others.

The presence of worms in goats at slaughter was determined by abomasal worm counts and only Haemonchus spp were recovered in the current study. Similarly, Haemonchus spp were found in the abomasa of sheep and goats in Ethiopia (Kumsa and Wossene, 2006). In contrast, abomasal worm counts in the goats in Ethiopia also recovered Trichostrongylus spp. Also, the Haemonchus prevalence in our study at 57 % and 43% is far less than the over 90% prevalence reported in studies in Ethiopia (Kumsa and Wossene, 2006). In agreement with the current study, Gatongi *et al.,* (1998) found Haemonchus spp to contribute over 80% of the worm burden in sheep and goats raised in semi-arid areas of Kenya. A Haemonchus worm burden of 500 -1000 in sheep less than 12 months of age is considered likely to be affecting health sufficiently to warrant treatment. Therefore the worm burden recorded in this study at less than 500 likely did not severely affect productivity in goats at slaughter age. Nevertheless, Haemonchus females are capable of laying large quantities of eggs resulting in heavy contamination of pasture, a significant risk factor for young goats. It is essential to carry out worm counts both in the abomasum and intestine in livestock since estimation of worm burden by faecal egg count techniques may be misleading by underestimating worm burden during the season when female worms are not laying eggs (Fritsche *et al.,* 1993). On the other hand, as was found out in the current study, some goats with a worm burden below detection, both in the abomasum and small intestine were positive for infection by the faecal egg counting technique suggesting that these goats were infected by few worms with a high egg output.

Conclusion

A simple method for preparing faecal samples for worm egg counting by McMaster technique achieves significantly higher egg counts per gram of faeces compared to the standard method. The worm egg count of goats reared under small scale communal set up is significant enough to impact productivity. Trichostrongylus spp of worms is most predominant in goats compared to other species of nemadotes based on larval culture. Haemonchus spp of worms is most predominant in the abomasum of goats.

Impact

Laboratories may use a simple method to prepare faecal samples for egg counting and expect reliable results. Small scale farmers should implement nematode control measures to increase productivity of their goat herds. And particular attention should be directed to Trichostrongylus spp and Haemonchus spp in the control of gastro-intestinal nematodes of goats in Botswana.

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References

Indre, D., Dărăbus, GH., Oprescu, I., Morariu, S., Mederle, N., Ilie, M.S., Măndiță, D.N., 2009. The identification of gastrointestinal nematodes species in sheep in five localities from Timis county. Lucrări Stiințifice Medicină Veterinară volume xlii (1), 94 -100.

Fritsche, T., Kaufmann, J., Pfister, K., 1993. Parasite spectrum and seasonal epidemiology of gastrointestinal nematodes of small ruminants in The Gambia. Veterinary Parasitology, 49,271-283.

Gatongi, P. M., Prichard, R. K., Ranjan, S., Gathuma, J. M., Munyua, W. K., Cheruiyot, H., Scott. M. E., 1998. Hypobiosis of Haemonchus contortus in natural infections of sheep and goats in a semi-arid area of Kenya. Veterinary Parasitology, 77, 49-61.

Kumsa, B., Wossene, A., 2006. Abomasal nematodes of small ruminants of Ogaden region, eastern Ethiopia: prevalence, worm burden and species composition. Revue De Medecine Veterinaire, 157 (12), 27-32.

Lyndal-Murphy, M., 1987. Diagnosis of anthelmintic resistance in sheep. Australian Standard Diagnostic Techniques for Animal Diseases, No. 46 pg 10-13.

Morgan, E. R., Coles, G. C., 2010. Nematode control practices on sheep farms following an information campaign aiming to delay anthelmintic resistance. Veterinary Record, 166,301-303.

Nsoso, S. J., Mosala, R., K. P., Ndebele, T., Ramabu, S. S., 2000. The prevalence of internal and external parasites in pigs of different ages and sexes in Southeast District, Botswana. Onderstepoort Journal of Veterinary Resesearch, 67, 217-220.

Rinaldi, L., Coles, G.C., Maurelli, M.P., Musella, V., Cringoli, G., 2011. Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. Veterinary Parasitology, 177, 345–352.

Ratanapob, N., Arunvipas, P., Kasemsuwan, S., Phimpraphai, W., Panneum, S., 2012. Prevalence and risk factors for intestinal parasite infection in goats raised in Nakhon Pathom Province, Thailand. Tropical Animal Health and Production, 44,741-745.

Segwagwe, B.V.E.and Ramabu, S.S., 1999. Causes of mortalities in sheep and goats in Botswana. In: Aganga AAA, Chabo RG, Kgosimore M, Letso M, Omphile UJ (eds), Enhancing Sheep and Goat Production in Botswana, Proceeding of the Sheep and Goat Workshop, Botswana College of Agriculture, Gaborone, Botswana, April 19 – 23, 1999, pp 224-229.

Stafford, K.A., Morgan, E.R., Coles, G.C., 2009. Weight-based targeted selective treatment of gastrointestinal nematodes in a commercial sheep flock. Veterinary Parasitology, 164, 59–65.

Tsotetsi, A. M., Njiro, S., T. Katsande, C., Moyo, G., Baloyi, F., Mpofu , J., 2013. Prevalence of gastrointestinal helminths and anthelmintic resistance on small-scale farms in Gauteng Province, South Africa. Tropical Animal Health and Production, 45,751-761.

van Wyk, J.A., Cabaret, J., Michael, L.M., 2004. Morphological identification of nematode larvae of small ruminants and cattle simplified. Veterinary Parasitology, 119, 277–306.

van Wyk, J. A, Mayhew, E., 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. Onderstepoort Journal of Veterinary Research, 80, (1) 1-14.

Vadlejch, J., Petrtýl, M., Zaichenko, I., Čadková, Z., Jankovská, I., Langrová, I., Moravec, M., 2011. Which McMaster egg counting technique is the most reliable? Parasitology Resesearch, 109, 1387–1394.

Sweeny, J. P.A., Ryan, U. M., Robertson, I. D., Niemeyer, D., Hunt, P. W., 2012. Development of a modified molecular diagnostic procedure for the identification and quantification of naturally occurring strongylid larvae on pastures. Veterinary Parasitology, 190, 467-481.

EPIDEMIOLOGY OF NEWCASTLE DISEASE VIRUS AMONG LOCAL CHICKENS OF WEST AND SOUTH-WEST REGIONS IN CAMEROON

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Abstract

Newcastle disease (ND) is one of the major constraints to poultry in most developing countries. It is a highly contagious and fatal disease caused by a virus of the family Paramyxoviridae. In other to evaluate the evidence of ND among village chicken, an epidemiological survey was carried out between September and October 2013 in 7 villages (Foumban, Bangang, Tole, Tiko, Muyuka and Muea) of two regions (West and South-West) in Cameroon. One hundred and thirty (130) blood samples were collected from the wing vein on free range local chicken. The blood was allow to clot at room temperature and the serum collected was kept in a freezer until analysis. Serological tests were done using the indirect ELISA test.

The overall seroprevalence was 32.30%, with 28.26 and 34.52% respectively for the West and South-West regions. In the West region, Foumban recorded the highest seroprevalence (34.37%) compared to Bangang (14.29%) while in the South-West region Tole has the highest seroprevalence (70%) followed in order by Tiko (38,7%), Muyuka (30%) and Muea (21,21%). Younger chickens (0-16 weeks) presented a lower seroprevalence (15.38%) than older ones (43.10%). Also, the mean antibody titer was lower in younger chickens (4782.92 unit/ml) than in older hens (7284.88units/ml). Considering the chicken phenotype, naked neck recorded the highest seroprevalence (40%) followed by the normal feathering chicken (38%), the crested (31.82%), the feathered tarsus (27.78%) and finally the crested-feathered tarsus (25%). The seroprevalence of Newcastle disease was not influenced by the vaccination as in the contrary of antibody titer.

Key words: epidemiology, Newcastle disease, local chicken, Cameroon

EPIDÉMIOLOGIE DU VIRUS DE LA MALADIE DE NEWCASTLE PARMI LES POULETS LOCAUX DES RÉGIONS OUEST ET SUD-OUEST DU CAMEROUN

Resume

La maladie de Newcastle est une contrainte majeure à la production des poules locales dans plusieurs pays en développent. C'est une maladie contagieuse causée par un virus de la famille des paramyxoviridae. Dans l'optique de mettre en évidence la maladie de Newcastle chez la poule locale, une étude a été menée dans les régions de l'Ouest et du Sud-Ouest Cameroun, entre les mois de Septembre et octobre 2013. Cent trente (130) échantillons de sang ont été collectes dans 7 villages (Foumban, Bangang, Tole, Tiko, Muyuka et Muea) sur des poules locales en divagation. Le sang était laissé à température ambiante jusqu'à coagulation et le sérum collecté dans des tubes eppendhorf était conservé au congélateur jusqu'à l'analyse sérologique.

La séroprévalence globale a été de 32,30%, avec 28,26 et 34,52% respectivement pour les régions Ouest et Sud-Ouest. Dans la région de l'Ouest, Foumban a enregistré la plus forte séroprévalence (34,37%) par rapport à Bangang (14,29%), tandis que dans la région Sud-ouest Tole a la séroprévalence la plus élevée (70%), suivi dans l'ordre par Tiko (38,7 %), Muyuka (30%) et Muea (21,21%). Les poules les plus jeunes (0-

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16 semaines) ont présenté la séroprévalence plus faible (15,38%) que les poules adultes (43,10%). En outre, le titre d'anticorps moyen est plus bas chez les poules plus jeunes (4782,92 unité / ml) que chez les poules âgées (7284,88units / ml). Considérant le phénotype, le cou nu a enregistré la plus forte séroprévalence (40%), suivie par le poulet à emplumement normal (38%), huppé (31,82%), le tarse emplumé (27,78%) et enfin le tarse emplumé - huppé (25%). La séroprévalence de la maladie de Newcastle n'a pas été influencé par la vaccination, contrairement au titre d'anticorps.

Mots clés: épidémiologie, maladie de Newcastle, poule locale, Cameroun

Introduction

In many developing countries, village poultry farming is widespread, especially in rural areas. Village poultry is predominant and practiced mainly by poor families. In Cameroon, the poultry population of about 35 million of birds has 80% of local poultry (Fotsa *et al.,* 2007). These animals play a very important role in socio-economic plan. Indeed, meat and eggs from chicken are the only source of protein for rural population, the income from poultry farming allows the family to support daily needs such as education of children, health, clothes and others (Copland and Alders, 2005).

Despite the importance of local chicken, its breeding in rural zone is still very neglected by public authorities, various organizations and the farmers themselves in favor of modern aviculture. Thus, it is exposed to problems such as lack of appropriate housing and food, theft, predation and infectious diseases (Alders, 2009; Dos Anjos, 2007). Among the diseases of local chicken, Newcastle disease (ND) has been identified as the major constraint in the production of village poultry (Alexander, 1991). Newcastle disease also called Rhaniket disease is a contagious and fatal disease caused by a paramyxovirus of the genus Avulavirus belonging to the family of Paramyxoviridae (OIE, 2012). It is endemic in many countries of the world, causing high economic losses due to high mortality and morbidity, decreased productivity and stress (Alexander, 2000). According to Alexander et al (2004), it can kill up to 80% of non-immunized chickens. Vaccination has been reported as the only means of protection against Newcastle disease (Orajaka et al, 1999). However, in order to establish an appropriate vaccination program and adequate control of ND in village poultry, it is necessary to know the immune status of local chicken in rural areas.

This study is an attempt to investigate the seroprevalence of Newcastle disease antibodies among free range local chicken for an appropriate control measure that will permit to farmers to fully enjoy their activity.

Materials and Methods

This study was conducted between September and October 2014 in 7 villages: Bangang and Foumban (West region), Muyuka, Muea, Tole and Tiko (South-west region). In each village, the families were first identified according to the good cooperation of the owners. Blood samples were collected in all the registered houses in the morning regardless of the number of chicken available.

We collected a total of 130 blood samples distributed in two villages of West region (Foumban and Bangang) and 4 villages in the South-west (Muyuka, Muea, Tole and Tiko). Note that the main obstacle at this point is the lack of cooperation of owners.

Two (2) ml of blood was collected at the wing vein with a syringe and placed in a sterile dry tube. The blood was allowed to clot at room temperature and the serum collected was kept in plastic tubes in a freezer up to serological examination. The indirect ELISA test was used to determine the antibody titers in serum samples, following the instructions of the kit manufacturer. A sample is positive when its antibody titer is > 396.

The chi-square test was used to compare the frequencies of the different parameters according to factors of variation. A significant level of 5% was used.

Results

General conditions for local chicken farming

In all the villages studied, village aviculture is practiced under conditions that are similar to those in others African countries. Animals generally come from local markets and sometimes donations. They are raised sometimes in together with turkeys or ducks. The minimum number of birds was 5 and a maximum of 24. The hens are left for free range during the day and only come back in the evening to be housed either in the family kitchen, in an empty room of the house, in a henhouse built with local materials or on a tree near the house. They feed by pecking in nature (plants, earthworms, insects ...) and generally drink waste water. In some families, grains are given to them every morning with food remaining.

ND is well known by populations through the description of various signs (nervous, respiratory and digestive) and devastation caused among poultry from September to November. From the appearance of the first signs of disease, the owners give to sick animals either tetracycline, garlic, aloe Vera, coal or metronidazole. This is directly in the mouth or into the drinking water. There is no preventive measures taken by farmers against ND or against any other pathology of local chicken. Yet the losses are significant and even integrated into their habits. Other difficulties are related to theft and predation (snake, hawk, dog ...).

Seroprevalence of Newcastle Disease virus in the local chicken population of West and South-West regions of Cameroon

The overall seroprevalence of ND was 32.30%, with 28.26 and 34.52% respectively in West and South-West (Table 1).

The seroprevalence of ND virus in the study villages is shown in Figure 1. It appears that ND virus is present in all the villages. In the West, the highest seroprevalence ($P<0.05$) was identified in Foumban (34.37%) against 14.29% in Bangang. In the South-West, Tole had the highest seroprevalence (70%), followed in order by Tiko (38.70%), Muyuka (30.00%) and Muea (21.21%). In the South-West, the seroprevalence of ND virus in Tole was significantly higher ($P<0.05$) than that of the 3 other villages that were significantly similar.

3) Seroprevalence of Newcastle Disease virus according to the age of local chickens

The seroprevalence of ND virus in local chicken according to age groups is shown in Figure 2. It appears that all age groups possess antibodies against Newcastle disease. However, the seroprevalence of ND antibodies in older animals (17 weeks and above) (43.10%) was significantly higher ($P<0.05$) than that of younger animals (15.38%). Also, the mean antibody titer in younger chicken (4792.92 units/ml) was significantly lower than that of older chickens (7284.88 units/ml).

4) Seroprevalence of Newcastle disease according to phenotype

Table 2 shows the distribution of seroprevalence of ND virus according to the phenotype of local chicken. It shows that the chickens of all encountered phenotypes possess antibodies against ND. The seroprevalence of ND in necked neck (40.00%) was significantly comparable to that of normal feathered chicken (38.00%) on one hand, and higher than those of crested (31.82%), feathered tarsus (27.78%) and of crested-feathered tarsus (25.00%) on the other hand. The last three being also comparable ($P<0.05$). Highest antibody titers were recorded in naked neck (27861.38 units/ml), followed in order by crested (4011.68 units/ml) and feathered tarsus (4282.36 units/

Table 1: Seroprevalence of ND in West and South-West Cameroon

Region	Number of animal collected	Positive samples	Seroprevalence (%)
West	46	13	28,26
South-West	84	29	34,52
Total	**130**	**42**	**32,30**

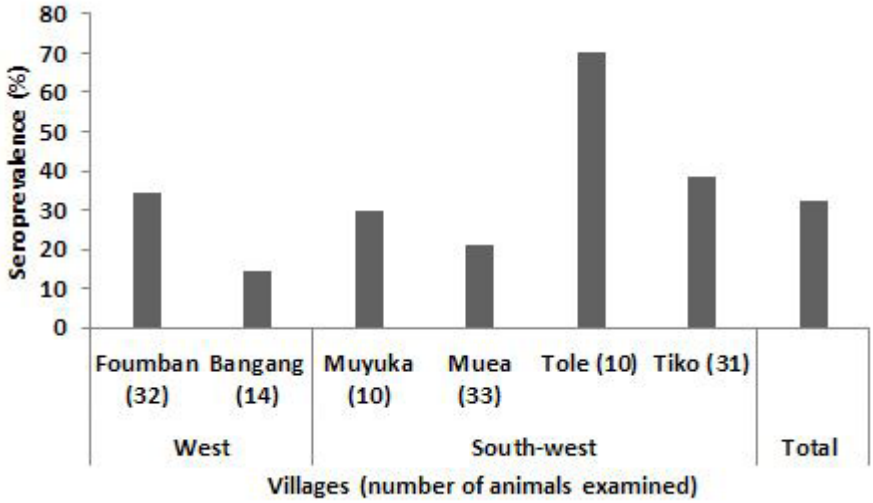

Figure 1: Seroprevalence of Newcastle Disease virus in the studied villages

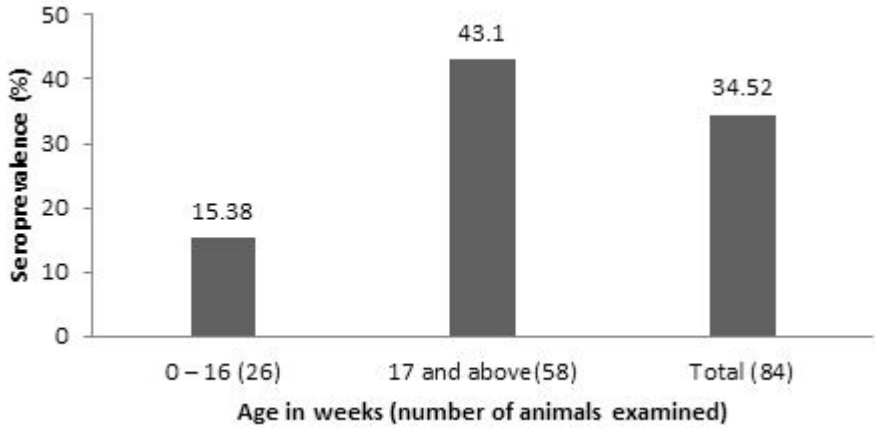

Figure 2: Seroprevalence of Newcastle disease in the local chicken according to age.

Table 2. Seroprevalence of Newcastle disease according to the phenotype of the local chicken

Phenotype	Total number of animals	Positive samples	Percentage (%)	Mean antibody titer (units/ml)
Normal	50	19	38,00[a]	8666,52[a]
Crested	22	7	31,82[b]	4011,68[b]
Feathered tarsus	18	5	27,78[b]	4282,36[b]
Naked neck	5	2	40,00[a]	27861,38[c]
Crested-feathered tarsus	4	1	25,00[b]	1188,5[d]
Total	**99**	**34**	**34,34**	**4601,04**

In the same column, values followed by the same letter are significantly comparable (P<0.05).

Table 3: Influence of vaccination on the seroprevalence of ND in the local chickens.

	Total number of animals	Positive samples	Percentage (%)	Mean antibody titer (units/ml)
Before vaccination	116	40	34,48	6398,6[a]
After vaccination	99	34	34,34	8184,12[a]

In the same column, values followed by the same letter are significantly comparable (P<0.05).

ml) with comparable antibody titers, the normal feathered (8666.52 units/ml) and finally the crested feathered tarsus which recorded the lowest titers (1188.50 units/ml).

5) Influence of vaccination against ND on the immune status of local chickens

Table 3 shows the seroprevalence of ND before and 21 days after vaccination. It is clear from this table that vaccination did not significantly affect the seroprevalence. Indeed, both before vaccination (34.48%) or 21 days after vaccination (34.34%), seroprevalence remained significantly similar (P<0.05). However, a comparison of mean antibody titers reveals that the antibody titer in animals after vaccination is higher.

Discussion

This study revealed the presence of antibodies against ND in the two regions studied, with 28.26 and 34.52% respectively in the West and Southwest regions. This implies that the ND virus is present in these village farms. Indeed, these animals have never been vaccinated, as stated by the farmers themselves, the presence of antibodies highlights natural infection with ND Virus. This result is similar to that of Nwankiti et al in 2010 (56.3%) obtained on local chickens in Nigeria as well as to that of Agbede et al in 1992 (48.88%) and Mai et al in 2014 (10.5%) obtained from local chickens in Cameroon. However, the overall prevalence obtained in this study (32.30%) is lower than those obtained by Nwankiti et al (2010) and Agbede et al (1992) on one hand, and higher than that of Mai et al (2014) on the other hand. These results confirm that ND remains a major constraint to the production of local chicken in most developing countries and even in some developed countries like France (Rauw et al, 2009).

In the Western Region, Foumban presented the highest seroprevalence. This could be related to the fact that the families visited in this village are very close to the city of Foumban which is an urban area with a significant concentration of modern farms. Similarly, the village Tole which had the highest prevalence in the South West (70%), is located near the village market and the tea plantations. Thus the movement of people and vehicles is very pronounced between these villages and the surrounding cities. Thus, failure in biosafety on people, vehicles, and even on food in modern urban farms would therefore contribute to continual introduction of virus in these farms (Alders and Spradbraw, 2000). Finally considerable variations in antibody titers from one animal to the other would be due to infection by several strains of variable virulence, or to the fact that the chickens were infected several times with the different strains of the ND virus (Grunder et al, 1988) or to genetic differences in chickens studied (Meulemans 1988; Keambou et al, 2013).

Youngest chicken (0-16 months) showed a significantly lower prevalence than that of animals over 16 weeks. This implies that younger animals are less provided with antibodies against ND than adults. This result is in contradiction with that of Mozafor et al (2010) who recorded highest seroprevalence among broilers between 0-6 weeks. This could be due to the fact that local chicken receiving no vaccination against ND, young animals are generally less immunized and it is the constant natural exposure to the ND virus that will allow them to produce antibodies. In the contrary, modern farms chicken as studied by Mozafor et al (2010) usually have a considerably level of maternal-derived antibodies.

The distribution of seroprevalence according to local chickens phenotypes showed that necked neck were most immune,

followed in order by the normal feathering chicken, the crested, the feathered tarsus and finally crested-feathered tarsus. This could be linked to genetic factors. Indeed, Keambou et al (2013) in a study of the influence of genetic type and sex on reproduction, growth, survival performance and thermal tolerance index observed that naked neck were most thermo-tolerant and feathered tarsus less heat-tolerant and most susceptible. In this study the seroprevalence decreases with the growing feather cover. This result might suggest a close relationship between the resistance to heat stress and resistance to infections in local chickens, what remains to be demonstrated.

Vaccination did not significantly influenced the seroprevalence of ND in free range local chicken. The response to vaccination was highly variable from one animal to another. This result is contrary to that of Ali and Bushra (2009) who obtained a significant increase in antibody levels in chickens 24 days after vaccination. This could be related to the fact that 21 days after vaccination, the vaccine used did not yet sufficiently induces the production of antibodies in animals. We could also think of the immunosuppressive effect exerted by some viruses such as infectious bursal disease virus, Chick anaemia virus and Marek`s disease virus (McMullin, 1985) and gastrointestinal parasites (Horning et al, 2003) on the effectiveness of a vaccine, or the presence of circulating antibodies at a high rate in some animals. Indeed, it has been shown in broilers that high levels of maternal antibodies would eliminate the production of antibodies induced by a vaccine. Finally, we will not exclude the fact that some vaccine protocols designed for the modern farms could not very suited for village farming systems.

Conclusion

This study revealed that Newcastle disease virus is circulating among local chickens population in Cameroon. Antibodies against Newcastle disease were detected in all the villages and the two regions studied. The seroprevalence of ND antibodies was influenced by age and phenotype of chickens. Younger chickens has lower antibody titers and seroprevalence of ND antibodies than older ones. Concerning the phenotype, crested feathered tarsus has the lowest seroprevalence and antibody titers while naked neck recorded the highest seroprevalence and antibody titers. Vaccination did not significantly affect the seroprevalence of ND antibodies after 21 days.

Acknowledgements

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References

Agbede G, Demey F, Verhulst A et Bell JG, 1992. Prevalence of Newcastle disease in traditional breeding facilities for chickens in Cameroon. Rev. Sci. Tech., 11 (3):805-811.

Alders C, 2009. Improving village chicken production: a manual for field workers and trainers. Canberra: Australian center for international agricultural research (ACIAR). ISBN 978 1 921531 (online).

Alders RG et Spradbrow P, 2000. La maladie de Newcastle dans les élevages avicoles villageois. Manuel de terrain. ACIAR

Alexander D J, 1991. Newcastle disease vaccines and other paramyxovirus infections. In: Calnek BW (Ed) Disease of poultry. Ames, Lowa state University press, Lowa. P 516-519.

Alexander D J, 2000. Newcastle disease and other avian paramyxoviruses. In: Revue Scientifique et Technique de l'OIE, pp443-462.

Alexander D J, Bell J G, Alders R G, 2004. A technology Review: Newcastle Disease. With special emphasis on its effect on village chickens. FAO Animal Production and Health Paper (FAO). 0254-6019, no 161.

Ali AS, Bushra HF, 2009. Efficacy evaluation of inactivated local vaccines against Newcastle disease in broilers. Iraqi J. Biotech. 8(1):412-418.

Copland J W and Alders C, 2005.The comparative advantages of village or smallholder poultry in rural development. In: village chickens, poverty alleviation and the sustainable control of Newcastle disease. Proceedings of an international conference, Dar es Salaam, Tanzania, October 5-7, 2005.Proceedings no 131. 11-14. Canberra: Australian Center for International Agricultural Research (ACIAR).

Dos Anjos F, 2007. The epidemiology of poultry disease, structure and importance of commercial and village based poultry industry in Mozambique. FAO report 2007.

Fotsa J-C, Bordas A, Rognon X, Tixier-Boichard M, Pone K D et Manjeli Y, 2007. Caractérisation des élevages et des poules locales et comparaison en station de leurs performances à celle d'une souche commerciale de type label au Cameroun. Septièmes journées de la recherche avicole, Tours, 2007.

Grundler G, Schmidt M and Djabakou K, 1988. Sérologie de la Maladie de Newcastle et de la salmonellose (S. gallinarum pullorum) chez la volaille de petites exploitations paysannes au Togo. Rev. Elev. Med. Vet. Pays Trop., 41(4) 327-328.

Horning G, Rasmussen A, permin A and Bisgaard M, 2003. Investigation of influence of helminths parasites on vaccination of chicken against Newcastle disease virus under village conditions. Tropical Animal Health and Production. 35:415-424.

Keambou T C, Hako B A, Bembide C, Ngono Emma P J and and Manjeli Y, 2013. Effects of type and sex on reproductive, growth, survival performance and thermal tolerance index of the local chicken (Gallus gallus) of the western highlands of Cameroon. International Journal of Poultry Sciences 12 (2):80-89, 2013.

Mai HM, Qadeers MA, Bawa IA, Sanusi M, Tayong KN, and Saidu I, 2014. Seroprevalence of Newcastle disease in local chicken in Mezam division of North-west Cameroon. Microbiology Research International.Vol2 (1) pp 9-12.

Meulemans G, 1988. Newcastle disease: control by vaccination. In: Newcastle disease (DJ Alexander, edit). Kluwer Academic oublisher, Norwell, Massachussets, 318-328.

Mozafor HKM, Yamin AMd, Ichiro Y, 2010. Antibody levels against Newcastle Disease Virus in chicken in Rajshahi and surrounding districts of Bangladesh. International Journal of Biology. Vol 2, no2:P102-106.

Njagi L W, 2008. Endemicity of Newcastle disease virus in village indigenous chicken and the role of carrier ducks. PhD thesis, University of Nairoby, Kenya.

Nwankiti O O, Ejekwolu A I, Ibrahim I, Ndako J A and Echeonwu G O N, 2010. Detection of serum antibody levels against Newcastle disease in local chicken in Bauchi Metropolis. Bauchi state, Nigeria. African Journal of Clinical and Experimental Microbiology 11(2): 95-101.

OIE (Office International des Epizoties), 2012. Newcastle disease.OIE Terrestrial Manual. France, Paris.

Orajaka LJE, Adene DF, Anene BM and Onuoha EA, 1999. Seroprevalence of Newcastle disease in local chicken from Southeast derived savannah zone of Nigeria. Revue Elevage médicine Vétérinaire Pays Tropicaux, 52(3-4), 185-188.

Rauw F, Gardin Y, Van Den Berg T and Lambrecht B, 2009. La vaccination contre la Maladie de Newcastle chez le poulet (Gallus gallus). Biotechnol. Agron. Soc. Environ. 13(4). 587-596.

AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2013

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The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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