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EFFECTS OF ENZYME ADDITIVE ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS OF CALVES FED FRESH GRASS AND HAY BASED DIETS.

Yusuf K O[1], Isah O A[1], Aderinboye R Y[1] and Fasae O A[2]
[1]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, P.M.B. 2240, Abeokuta, Nigeria
[2]Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria.

Abstract

This study was conducted to determine the effects of an exogenous fibrolytic enzyme (ROXAZYME G®) on blood parameters of calves fed fresh grass and grass hay-based diets. Chemical composition of concentrate, fresh grass and grass-hay were determined. Sixteen crossbred (N'dama × Muturu) calves were subjected to two feeding trials that lasted 84 days each in the wet season and dry season. Fresh grass (*Panicum maximum*) was the basal diet in the wet season while, the hay was the basal diet in the dry season. Four concentrate diets were formulated with enzyme inclusion at 0, 50, 100 and 150 mg/kg to form four dietary treatments (T1, T2, T3, T4), with four animals per treatment in a completely randomized design. Results showed that fresh grass had higher crude protein (CP), ether extract and ash than hay. However, the hay had higher dry matter, crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents. The calcium and phosphorus contents of the concentrate diets were higher than that of fresh grass and hay. At the end of the first experiment, Packed cell volume (PCV), Haemoglobin (Hb) and Red blood cell (RBC) values were highest ($P<0.05$) in T3 and lowest in T1 (control). White blood cell (WBC) and neutrophil counts were highest in T1. *Transaminase enzymes*: AST and ALT were highest ($P<0.05$) in T4. In the second trial, the diets influenced ($P<0.05$) the WBC, lymphocyte, total protein and AST. The WBC was also highest in T1 while, lymphocyte was highest in T2. Total protein was highest in T3 and AST was highest in T4. It was therefore, concluded that the inclusion of ROXAZYME G® up to 150 mg/kg DM in the diets of calves improved their blood parameters without any deleterious effect.

Keywords: blood parameters, dry season Panicum maximum, ROXAZYME G®, wet season,

LES EFFETS DE L'ENZYME ADDITIF SUR LES PARAMÈTRES HÉMATOLOGIQUES ET BIOCHIMIQUES SÉRIQUES DES VEAUX NOURRIS À L'HERBE FRAICHE ET AUX RÉGIMES À BASE DE FOIN.

Résumé

Cette étude a été menée afin de déterminer les effets d'une enzyme fibrolytique exogène (ROXAZYME G®) sur les paramètres sanguins des veaux nourris à des rations à base d'herbe fraîche et de foin. Seize veaux croisés (N'dama × Muturu) ont été soumis à deux essais d'alimentation qui ont duré 84 jours chacun pendant la saison humide et la saison sèche. L'herbe fraîche (*Panicum maximum*) était l'alimentation de base pendant la saison humide tandis que le foin était l'alimentation de base en saison sèche. Quatre concentrés alimentaires formulés à partir de l'inclusion d'enzymes à 0, 50, 100 et 150 mg / kg ont été utilisés pour quatre traitements alimentaires (T1, T2, T3, T4), avec quatre animaux par traitement dans un dispositif entièrement aléatoire. Les résultats ont montré que l'herbe fraîche contenait plus de protéines brutes (PB), d'extrait d'éther et de cendres que le foin. Cependant, le foin avait des teneurs en matières sèches, en fibres brutes, en fibres détersives neutres (FDN), en fibres détersives acides (FDA) et en lignines détergentes acides (LDA) plus élevées. Le contenu en calcium et en phosphore des concentrés était plus élevé que celui de l'herbe fraîche et du foin. À la fin de la première expérience, l'hématocrite

Corresponding author email: yomowumi@gmail.com

(HE), l'hémoglobine (Hb) et les globules rouges (GR) étaient les plus élevées (P <0,05) dans le T3 et les plus faibles dans le T1 (témoin). Les taux de globules blancs (TGB) et de neutrophiles étaient les plus élevés dans le T1. Les enzymes transaminase: AST et ALT étaient les plus élevés (P <0.05) dans le T4. Dans le deuxième essai, les régimes ont eu un effet (P <0,05) le TGB, les lymphocytes, la protéine totale et l'AST. Le TGB était le plus élevé dans le T1 tandis que le lymphocyte était le plus élevé dans le T2. La protéine totale était la plus élevée dans le T3 et l'AST était la plus élevée dans le T4. Il a donc été conclu que l'inclusion de ROXAZYME G® jusqu'à 150 mg / kg de MS dans l'alimentation des veaux améliorait leurs paramètres sanguins sans aucun effet délétère.

Mots clés: les paramètres sanguins, la saison sèche *Panicum maximum*, ROXAZYME G®, saison humide.

Introduction

The bulk of cattle, sheep and goats in developing countries are produced on natural grassland (Alokan 1998). Thus, the supply of good quality forage throughout the year is necessary if a reasonable level of production is to be maintained from ruminant animals. However, changes in weather conditions have been reported to affect the nutrient quality of forages consumed by ruminant livestock, especially during the six months dry season, with the crude protein content of grasses falling below 6% (Onwuka and Olatunji 1996). Invariably the animals lose the weight that they have accrued when grasses were green.

Thus, the productivity of the stock is limited by non-availability of good pasture as well as by the low quantity, quality and regularity of supplements provided (Taiwo *et al.,* 2005). Attempts have been made by animal nutritionists to supplement dry season grazing with browse plants and agro-industrial by-products. These were, however, limited by their content of anti-nutritional factors and low digestibility. The use of exogenous fibrolytic enzymes hold promise as a means of increasing forage utilization, and improving the production efficiency of ruminants (Beauchemin *et al.,* 2003).

Blood has been identified as a transport medium through which hormones and metabolites are transported. It also provides a means through which thermo-regulation and general homeostasis take place (Duke 1975). According to Onifade and Babatunde (1993), blood examination is a good way of assessing the health status of an animal and this plays an important role in the physiological, nutritional and pathological structure of an organism. Research has demonstrated that, supplementing dairy cow and feedlot cattle diets with fibre-degrading enzymes has significant potential to improve feed utilization and animal performance. But, there is paucity of information on the influence of exogenous enzymes on blood. Hence, the objective of this study was to examine the blood parameters of calves fed fresh grass and hay based diets with enzyme additive.

Materials and Methods

Study Site

The study was conducted at the Cattle Unit of the Teaching and Research Farm Directorate, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Ogun State is in the rainforest zone of South West Nigeria. The area has an annual mean temperature of 34.7°C, a relative humidity of 82% and an annual mean rainfall of 1,037 mm. It is about 70 m above sea level and lies on latitude 7°5'-7°8'N and longitude 3°11.2'E.

Collection and Preparation of Samples for Analyses

The grass (*Panicum maximum*) which had earlier been cut back in sequence was harvested from eight weeks old re-growth of the ruminant paddock. The samples were oven-dried at 60oC to constant weight to determine the dry matter content. The dried samples were then milled and sieved to 1.0 mm particle size and stored in an air tight container till required for analysis. Four concentrate diets were compounded with ROXAZYME G2® (an exogenous fibrolytic enzyme consisting of cellulase, xylanase and beta-glucanase)

supplemented at 0, 50, 100 and 150 mg/kg dry matter (Table 1). The P. maximum hay was prepared by curing freshly harvested P. maximum on a clean concrete floor by sun drying for 2-3 day-lights. The hay was loosely stored in sacks pending usage.

Chemical Analyses

Crude protein, crude fibre, ether extract and ash contents of the fresh grass, hay and the concentrate diet were determined according to AOAC (2000). The fibre factions; neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were also determined (Van Soest *et al.,* 1991).

Cellulose and hemicellulose were derived from NDF, ADF and ADL by simple calculation as follows: Hemicellulose = NDF - ADF, Cellulose = ADF - ADL.

Calcium was determined using Jenway flame photometer (Model PFP7) while phosphorus was assayed according to the Association of official Analytical Chemists (AOAC 2000) procedures.

Experimental Diets

Two independent studies were carried out. The basal diet for the first study was fresh P. maximum supplemented with concentrate diet while P. maximum hay was the basal diet in the second study. The fresh grass was fed wilted. Four concentrate diets were formulated with ROXAZYME G2® included at levels of 0, 50 100 and 150 mg/kg dry matter (Table 1). The enzyme was purchased from a commercial dealer. The concentrate supplement was fed at 40% of the animal daily feed requirement while the forage diet made up the remaining 60%. Experimental animals were fed at 5% of their body weights with the concentrate supplement offered first at 8.00 a.m. and the basal diet an hour after in separate feeding troughs. The experimental diets were outlined as follows:

Fresh grass or hay +Concentrate+0 mg/kg ROXAZYME G2®

Fresh grass or hay +Concentrate+50 mg/kg ROXAZYME G2®

Fresh grass or hay +Concentrate+100 mg/kg ROXAZYME G2®

Fresh grass or hay +Concentrate+150 mg/kg ROXAZYME G2®

Experimental animals and their Management

Sixteen (16) crossbred (N'dama x Muturu) female calves aged 7-9 months and weighing 60 to 70 kg were used for the first study while, the animals used for the second study were aged 12-14 months and weighed 70-80 kg.

The calves were housed in well ventilated individual pens. The pens were thoroughly washed and disinfected. The animals were de-wormed with Albendazole® 2.5% oral suspension (Anthelmintics) at 1 mL/10 kg body weight and treated against ectoparasites with Cypermethrin® Pour-on at 1 mL/10 kg body weight before allotting them to individual pens. There were four animals per treatment and were allowed an adaptation period of two weeks prior to commencement of the experiment during which they were maintained on fresh *Panicum maximum* and concentrate supplement. The experiment lasted 84 days (12 weeks) for each of the studies.

Blood analysis

Prior to the commencement of each of the experiments, 10 mL of blood samples were collected from each of the animals in each treatment, in well labeled bottles. This was repeated at the end of each of the experiments. The blood sampling was in the morning before feeding via jugular vein puncture using hypodermic syringe. First 5 mL each of the blood samples weres drawn into bottles containing EDTA (ethylenediaminetetraacetate) anti-coagulant for the determination of packed cell volume (PCV), haemoglobin concentration (HB), red blood cell (RBC) and white blood cell (WBC), while another 5 mL was left in the syringe for harvesting of serum for determination of total protein, glucose, urea,

Table 1: Gross composition of concentrate supplements (g/kg)

Ingredients	Levels of inclusion of enzyme (mg/kg)			
	T_1(0)	T_2(50)	T_3(100)	T_4(150)
Wheat offal	650	650	650	650
Dried brewers' grain	300	300	300	300
Blood meal	20	20	20	20
Bone meal	20	20	20	20
Common salt	10	10	10	10
Enzyme	-	+	++	+++
Total	**1000**	**1000**	**1000**	**1000**

Source: *Yusuf et al. (2013); +: Enzyme additive*
T_1, T_2 T_3, and T_4 represent enzyme inclusion levels at 0, 50, 100 and 150 mg/kg of the concentrate

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT).

Statistical Analysis

Data collected from each of the experiment were subjected to Analysis of Variance (ANOVA) as Completely Randomized Design and where variations existed, means were compared using Duncan's Multiple Range Test with SAS package (SAS 1995). The mathematical model of the experiment is as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y_{ij} = Observed value of dependent variables; μ = Population mean; T_i = Treatment effect, and ε_{ij} = Random residual error.

Results

Table 2 shows the chemical composition of fresh *Panicum maximum* (fresh grass), P. maximum hay and concentrate diet. Variations existed in all the values obtained for fresh grass and hay. The grass hay had the highest (P<0.05) dry matter (DM), NDF, ADF, ADL and cellulose (88.50%, 75.75%, 60.00%, 28.50% and 31.00%, respectively) while, fresh P. maximum had the highest crude protein (CP), ether extract (EE), ash, hemicelluloses as well as Ca and P as 14.20%, 6.50%, 11.50%, 18.40%, 0.39% and 0.25%, respectively).

The haematological parameters of calves fed fresh grass based diets at the start and end of the experiment are presented in Table 3. The diets influenced (P<0.05) the PCV, Hb, WBC, RBC, Neutrophil and Lymphocyte. The PCV, Hb and RBC increased by the end of the experiment. The PCV ranged from 31.25-33.75% at the start of the experiment to 32.00-35.00% at the end of the experiment with T3 having the highest (P<0.05) value and TI recording the lowest value. The Hb and RBC values also followed the same trend. However, the Hb and RBC values reduced at T4 at the end of the experiment. The WBC reduced from a range of 9.83-11.06 to 8.05-10.50g/dL with T1 recording the highest (P<0.05) value and the least value obtained at T2. Neutrophil also increased with the highest value (37.50%) obtained at T2. Lymphocyte was highest (67.50%) for calves in T3 and lowest (56.50%) in T1.

Table 4 shows the serum biochemical parameters of calves fed fresh grass based diets. The serum glucose increased from a range of 56.80-69.58 mg/dL at the start of the experiment to 64.75-68.95 mg/dL at the end of the experiment with the highest value obtained at T2. Serum total protein was highest at T3 (67.80 g/dL) and lowest at T1 (61.05 g/dL). Serum urea decreased across the experiment from a range of 18.75-23.90 mg/dL at the start of the experiment to 13.80-16.60 mg/dL at the end with T1 having the lowest value and T2 was having the highest value. The AST and ALT were affected (P<0.05) by the experimental diets. The AST was highest at T2 (51.20 iμ/L) and lowest at T1 (36.75 iμ/l) while ALT was highest at T4 (14.00 iμ/l) and lowest at T1 (9.20 iμ/l).

Table 2: Chemical composition of experimental diet components (%)

Parameters	Fresh Grass	*Hay	*Concentrate
Dry Matter	65.50	88.50	70.50
Crude Protein	14.20	7.60	15.75
Crude Fibre	10.52	14.40	13.50
Ether Extract	6.50	1.73	6.60
Ash	11.50	4.25	5.44
Neutral Detergent Fibre	65.60	75.75	56.40
Acid Detergent Fibre	47.60	60.00	30.00
Acid Detergent Lignin	20.00	28.50	14.85
Cellulose	27.20	31.50	15.15
Hemicellulose	18.40	15.75	26.40
Calcium	0.39	0.10	1.35
Phosphorus	0.25	0.05	1.54

**Yusuf et al. (2013)*

The haematological parameters of calves fed hay-based diets at the start and end of experiment are presented in Table 5. The WBC and Lymphocyte were influenced ($P<0.05$) by the diets. The WBC was highest (15.83×109/L) in T1 and lowest (9.15×109/L) in T2. However, there was a reduction in the RBC values, from a range of 9.62-10.07x1012 g/L to 7.71-8.78x1012 g/L. The serum glucose increased by the end of experiment from a range of 57.55-62.78 mg/dL to 76.30-73.70 mg/dL. Lymphocyte value was highest for calves at T2 (65.50%) and lowest for calves at T4 (51.00%).

The diets also influenced ($P<0.05$) total protein and AST at the end of experiment (Table 6). Serum total protein was highest in T3 (73.95 g/dL) and lowest in T1 (64.85 g/dL) at the end of the experiment. The AST increased across treatments with the highest value obtained in T4 (48.00 iμ/L) and lowest value obtained in T1 (39.85 iμ/L).

Discussion

The results indicate that, fresh grass recorded higher crude protein, ether extract, ash, calcium and phosphorus contents than the grass hay studied. This is an indication that, with sun-drying, these nutrients reduced. The fibre fractions of fresh grass, hay and the concentrate show that the diets possessed above the minimum fibre content (Crude fibre-17% and NDF-30%), which is very essential in ruminant feeding for adequate rumination (Jacobs and Hangreaves 2012).

The blood parameters of calves fed fresh grass based diet at the start and end of the experiment were within the normal range reported by some authors. There were no significant differences in almost all the parameters measured at the start of the experiment unlike at the end of the experiment. The packed cell volume (PCV) of the calves at the end of the experiment was within the normal range of 33-60% reported for cattle by Puls (1994). The red blood cell (RBC) count was higher by the end of the experiment except in T4. No reason can be adduced for this from the experiment. However the value obtained is in line with what was obtained for cattle in Nigeria (Olusanya *et al.,* 1976). The total white blood cell (WBC) count was higher and was within the range of 6.8-20.1 reported by Daramola et al (2005). Variations in PCV, Hb, and WBC agree with the values reported by Ajayi et al. (2005).

The blood parameters of calves fed hay based diets were also within the normal range reported by previous authors. The PCV

Table 3: Haematological Parameters of Calves fed fresh grass based diets

Parameters	At the start of experiment					At the end of experiment Levels of inclusion of enzyme (mg/kg)				
	*Normal Values	T_1	T_2	T_3	T_4	SEM	T_1(0)	T_2(50)	T_3(100)	T_4(150)	SEM
PCV (%)	33-60	31.25	32.25	32.75	33.75	0.67	32.00^{c}	34.50^{ab}	35.00^{a}	34.00^{b}	0.34
Hb (g/dl)	8-15	10.58	10.93	10.98	11.48	0.23	10.95^{c}	11.55^{b}	12.60^{a}	10.90^{c}	0.21
WBC (×109/l)	4-12	9.83	10.50	11.06	11.04	0.56	0.50^{a}	8.63^{c}	9.18^{b}	8.05^{d}	0.27
RBC (×1012/l)	5-10	6.64	6.81	7.08	7.16	0.13	6.90^{c}	7.36^{b}	7.75^{a}	6.95^{c}	1.10
Neutrophil (%)	10-45	33.25	30.25	34.75	31.25	1.45	37.50^{a}	30.50^{c}	35.50^{b}	35.00^{b}	1.27
Lymphocyte (%)	45-75	60.50	68.75	66.75	66.75	1.82	56.50^{b}	61.50^{a}	67.50^{a}	63.50^{a}	1.19
Eosinophil (%)	0.08-2.4	0.50	0.25	0.00	0.50	0.17	0.50	0.00	0.00	0.25	0.13
Monocyte (%)	0.025-0.85	0.00	0.75	0.00	0.00	0.14	0.50	0.00	0.00	0.00	0.20
Basophil (%)	0.0-0.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^{abc} = Means in the same row having different superscripts are significant (P<0.05)
SEM – Standard Error of Mean
**Normal values according to Fraser and Mays (1986)*

Table 4: Serum Biochemical Parameters of Calves fed fresh Grass based diets

Parameters	At the start of experiment					At the end of experiment Levels of inclusion of enzyme (mg/kg)				
	*Normal Values	T_1	T_2	T_3	T_4	SEM	T_1(0)	T_2(50)	T_3(100)	T_4(150)	SEM
Glucose (mg/dl)	40-80	64.13	69.58	60.28	56.80	2.28	68.35	68.95	65.25	64.75	0.56
Total Protein (g/dl)	59-88	75.10^{a}	$66 .30^{b}$	61.78^{b}	1.96	59.58^{b}	67.10	61.05	64.80	67.80	0.79
Urea (mg/dl)	10-20	23.90	18.75	23.55	23.00	0.94	13.80	16.60	14.30	14.70	0.32
AST (lμ/l)	31-70	36.65	34.90	47.75	29.30	3.04	36.75^{c}	51.20a	50.00^{b}	51.00a	1.83
ALT (lμ/l)	5-15	14.15^{a}	7.75^{b}	10.68^{ab}	9.15b	0.88	9.20^{d}	12.35c	12.85^{b}	14.00a	0.82

^{abc} = Means in the same row having different superscripts are significant (P<0.05)
SEM – Standard Error of Mean
**Normal values according to Fraser and Mays (1986)*

Table 5: Haematological Parameters of Calves fed hay based diets

Parameters	At the start of experiment						At the end of experiment Levels of inclusion of enzyme (mg/kg)			
	*Normal Values	T_1	T_2	T_3	T_4	SEM	T_1(0)	T_2(50)	T_3(100)	T_4(150)	SEM
PCV (%)	33-60	35.25	33.00	35.00	33.25	1.15	34.50	39.00	38.50	37.50	0.92
Hb (g/dl)	8-15	12.68	11.78	11.83	11.78	0.21	10.95	11.55	12.60	10.90	0.32
WBC ($\times 10^9$/l)	4-12	14.13	13.68	13.50	14.60	0.40	15.83^{a}	9.15^{b}	12.40^{ab}	10.50^{b}	0.83
RBC ($\times 10^{12}$/l)	5-10	10.07	9.95	9.74	9.62	0.39	7.71	8.78	8.35	8.54	0.20
Neutrophil (%)	10-45	26.73	26.75	26.40	28.56	1.14	40.50	32.50	37.00	40.50	0.99
Lymphocyte (%)	45-75	57.98^{b}	68.43^{a}	67.35^{a}	64.33^{ab}	1.53	56.00^{ab}	65.50^{a}	59.00^{ab}	51.00^{c}	1.90
Eosinophil (%)	0.08-2.4	0.25	0.00	0.50	0.50	0.06	2.00	1.00	2.00	1.50	0.45
Monocyte (%)	0.025-0.85	0.70	0.65	0.62	0.70	0.01	0.50	1.00	1.50	1.00	0.29
Basophil (%)	0.0-0.3	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.00	0.18

^{abc} = Means in the same row having different superscripts are significant (P<0.05)
SEM – Standard Error of Mean
**Normal values according to Fraser and Mays (1986)*

Table 6: Serum Biochemical Parameters of Calves fed hay based diets

Parameters	At the start of experiment						At the end of experiment Levels of inclusion of enzyme (mg/kg)			
	*Normal Values	T_1	T_2	T_3	T_4	SEM	T_1(0)	T_2(50)	T_3(100)	T_4(150)	SEM
Glucose (mg/dl)	40-80	62.78	58.10	62.68	57.55	1.22	73.70	71.35	76.30	73.70	1.27
Total Protein (g/dl)	59-88	73.60	77.10	75.28	71.90	0.87	64.85^{c}	71.50^{b}	73.95^{a}	71.70^{b}	1.03
Urea (mg/dl)	10-20	23.15	21.18	21.38	23.30	0.29	19.95	20.00	19.30	19.70	0.03
AST (Iμ/l)	31-70	34.03^{a}	27.73^{c}	31.70^{ab}	30.00^{bc}	0.81	39.85^{c}	41.05^{c}	44.40^{b}	48.00^{a}	0.96
ALT (Iμ/l)	5-15	20.78^{a}	18.05^{ab}	17.40^{b}	18.33^{ab}	0.56	13.40	14.20	14.60	14.00	0.13

^{abc} = Means in the same row having different superscripts are significant (P<0.05)
SEM – Standard Error of Mean
**Normal values according to Fraser and Mays (1986)*

and Hb values were not significantly influenced by the diets but, recorded numerical increase. However, the PCV values obtained (34.50-39.00%) fell within the range 33-60% reported by Puls (1994). The values therefore, were indicative of a good nutritional status of the calves. The values obtained for Hb in all the diets was a confirmation of protein level and quality, which is an indication that the diets supplied the animal the required level of protein. Pellet and Young (1980) confirmed that haemoglobin levels are, positively, correlated with protein quality and level in the diets. Udoh (1987) also reported that nutrition is the most important factor affecting Hb levels in the blood.

Muturu and Ndama cattle seem to possess protective system, providing a rapid and potent defense against any infectious agent and this is probably the physiological basis for the adaptation of this animal to this ecological zone characterized with prevalence of diseases. In calves like other ruminants, there are more lymphocytes and neutrophils in circulation (Olusanya *et al.,* 1976). This suggests the existence of a well developed immune system in calves. The variations in lymphocytes agree with those reported by Ajayi et al. (2005). On the other hand, high neutrophil values were obtained for the grass hay fed calves. Excessively high neutrophil is found during infection, stress condition or conditions induced by steroids (Oso 2007). The higher level of neutrophil was generated by livestock in a bid to fight against foreign bodies and this may also have been responsible for the higher values of WBC recorded. However, there was no record of illness throughout the experiment.

The serum glucose concentration of the calves was in line with the range of 55-95 mg/dL reported by Edward et al. (1996). The high level of blood glucose at the end of the experiment, especially, in T4 (150 mg/kg enzyme inclusion level) could be attributed to high level of sugar in the blood. Enough glucose is present in the system of the animals. According to Edward et al (1996), a continuous supply of glucose is necessary as a source of energy, especially, for the nervous system and the erythrocytes. Also below critical blood glucose concentration (48 mg/dl for goats) there is brain dysfunction, which can lead to coma and death (Wikipedia 2011).

In this study, the four treatments were statistically not different in terms of their total protein (TP) level in the serum although, the TP levels fell within the normal range of 59-88 g/L (Puls 1994). Serum proteins are important in osmotic regulation, immunity and transport of several substances in the animal body (Jain, 1986). The urea levels of the diets were similar and within the normal range (10-20 mg/dl) reported although, they were lower than the recorded values at the commencement of the study. This difference could be related to the quantity and quality of dietary protein, which are important determinants of circulating level of urea since, the ingestion of large amounts of readily absorbable protein produces substantial rise in blood urea nitrogen (Ronald 1975).

The higher values obtained for serum total protein of the calves on treated grass hay diets were indicative of good performance characteristics of the calves. Iyayi and Tewe (1998) reported a high correlation between serum total protein and performance in livestock. The values reported for serum urea (19.75-21.00%) are higher than the value of 19.0 mg/dL reported by Taiwo and Ogunsanmi (2003). Enzymes are protein catalysts, mostly, present in living cells and are constantly and rapidly degraded although renewed by new synthesis (Coles 1986).

The Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) values were also influenced by the dietary treatments. The AST values obtained increased across treatments and were in line with the value (12-38 and 20.9±1.2) reported by Daramola et al (2005) and Tambuwa *et al.,* (2002), respectively. The ALT values were within normal range reported and increased except in T1 where there was a reduction. There were no significant differences in the ALT values obtained. The AST values of T1 and T2 were not significantly different ($P>0.05$) but lower than that of T3 and T4. This could be an indication that the test diets did not differ in their effect on enzyme secretion mechanism.

The best enzyme secretion was obtained at T4 (150mg/kg enzyme inclusion level). However, values reported agree with those reported by Daramola *et al.,* (2005). *Transaminase enzymes* are those, mostly, responsible for the synthesis of non-essential amino acids through the process known as transamination (Carola *et al.,* 1990).

Conclusion

The haematological and serum biochemical parameters of the calves fed fresh *Panicum maximum* and *Panicum maximum* hay based diets with enzyme additive from this experiment were indicative of a good nutritional status of the animals. The inclusion of exogenous fibrolytic enzyme in the diet of the calves up to 150 mg/kg DM improved the PCV, Hb, ALT and AST when fresh grass form the basal diet and, AST and total protein when hay was the basal diet while reducing the WBC counts in both experiment.

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

Ewuola EO* Oyedele DM and Akinyemi DE,
Animal Physiology Laboratory, Department of Animal Science,
University of Ibadan, Ibadan, Nigeria.

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

LA MORPHOMÉTRIE GÉNITALE ET LES CARACTÉRISTIQUES TESTICULAIRES DES CAILLES BLANCHES JAPONAISES MÂLES À TROIS GROUPES D'ÂGES DIFFÉRENTS.

Résumé

Une expérience a été menee pour évaluer la morphométrie génitale des cailles blanches japonaises mâles de trois groupes d'âge différents. Cinquante-quatre mâles de cailles japonaises étaient répartis en 3 groupes de traitement (pubertaire, mature et adulte) dans un dispositif entièrement aléatoire. Les pubertaires (7-10 semaines), les matures (15-20 semaines) et les adultes (≥ 24 semaines). Le poids initial des cailles avait été pris. Tous les animaux abattus et les organes soigneusement excisés. La longueur totale du tractus reproducteur et les sections du tractus des cailles mâles était prélevée. On a déterminé le poids total des voies reproductrices, du poids testiculaire droit et gauche, de l'épididyme droit et gauche, du diamètre testiculaire droit et gauche et de la circonférence. Le poids du tractus génital masculin des cailles blanches japonaises était similaire dans les différents groupes d'âge. La longueur de l'appareil génital était significativement plus élevée dans le groupe pubertaire que chez les matures et les adultes. La circonférence testiculaire et le diamètre des cailles japonaises blanches à la puberté était significativement ($P <0,05$) plus élevé qu'à l'âge adulte. En conclusion, les cailles pubertaires mâles ont des voies reproductrices bien développées et pourraient donc avoir un potentiel de capacité reproductive élevée semblable à des âges physiologiques ultérieurs. Les agriculteurs peuvent faire plus d'activité de reproduction lorsque les oiseaux sont à l'âge pubertaire.

Mots clés: Tractus reproducteur, caille blanche, caractéristiques testiculaires, épididyme

*Corresponding author email: eoewuola@gmail.com

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).

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

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

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 (cm3)	1.94±0.64	1.67±0.49	1.50±0.86
Left Testis Volume (cm3)	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

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 □-endorphin and met-enkephalin inhibited GnRH-I 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 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

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 (cm3)	Paired testes vol (cm3)	Right testis C	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	.047	.044
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.*

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.

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.

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GROWTH PERFORMANCE, HAEMATOLOGY AND COST BENEFIT OF GROWING RABBITS REARED ON DIFFERENT FEED ACCESS TIMES AND RESTRICTION DURATIONS

Obasa, OA1*, Adeyemi, OA1, Akinola, OS1 and Olaifa, RO1
1Department of Animal Production and Health, 2Department of Animal Nutrition,
Federal University of Agriculture, Abeokuta, Nigeria.

Abstract

Sixty growing rabbits of mixed breeds and sexes were used for 10 weeks in a 4 x 3 factorial experimental design to test for the effect of different feed access times (2, 4, 6 and 24 h) and different restriction durations (2, 4 and 6 wk) on the performance, haematological parameters and cost benefits of growing rabbits. Data obtained were subject to a 2–way analysis of variance. Results showed significantly higher ($p<0.05$) final weight in rabbits maintained on 24-h feed access time (1875.4g), 6 h feed access time for 2-wk restriction duration (1822.0g), 6-h feed access time for 4-wk restriction duration (1821.0g) and 4 h-feed access time for 2-wk restriction duration (1828.0g). Growing rabbits on 24-h feed access time (control) recorded a higher ($p<0.05$) feed intake when compared with other treatments. Feed conversion ratio and mortality were not significantly affected ($p>0.05$) across the feed access time and restriction duration. White blood cell was higher in growing rabbits on 2-h feed access time for 6 wk duration of restriction while all other parameters measured for haematology were not significantly affected by the feed access time and restriction duration. Total cost of feed consumed was highest in growing rabbits maintained on 24-h feed access time. Cost of feed per kg weight gain was not significantly influenced across the feed access times and the restriction durations. It was concluded that for a reduced cost of feeding without an adverse effect on the performance and haematological profile, growing rabbits should be raised on not less than 4-h feed access time for 2-wk restriction duration.

Key words: Feed access time, feed restriction, haematology and cost

LA PERFORMANCE DE CROISSANCE, L'HÉMATOLOGIE ET LA RENTABILITÉ DE LA CROISSANCE DES LAPINS ÉLEVÉS AVEC LES DIFFÉRENTS TEMPS D'ACCÈS À L'ALIMENTATION ET LES DURÉES DE RESTRICTION

Résumé

Soixante lapins en croissance de races et de sexes mixtes ont été utilisés dans un dispositif expérimental factoriel 4 x 3 pendant 10 semaines pour tester l'effet des différents temps d'accès (2, 4, 6 et 24 h) et des différentes durées de restriction (2, 4 et 6 semaines) sur les performances, les paramètres hématologiques et les rentabilités des lapins en croissance. Les données obtenues ont fait l'objet d'une analyse de variance bidirectionnelle. Les résultats ont montré un poids final significativement plus élevé ($p <0,05$) chez des lapins maintenus pendant un temps d'accès de 24 h (1875,4 g), un temps d'accès de 6 h pour une durée de restriction de 2 semaines (1822,0 g), un temps d'accès de 6 h pour 4 semaines de durée de restriction (1821,0g) et 4 h-temps d'accès à l'alimentation pendant 2 semaines de durée de restriction (1828,0g). Les lapins en croissance ayant un temps d'accès à l'alimentation de 24 heures (témoin) ont enregistré une consommation d'aliments plus élevée ($p <0,05$) par rapport à d'autres traitements. Le taux de conversion alimentaire et la mortalité n'ont pas été significativement affectés ($p> 0,05$) à travers le temps d'accès et la durée de restriction. Le nombre de globules blancs était plus élevé chez les lapins en croissance, avec un temps d'accès de 2 h pour une durée de 6 semaines de restriction, tandis que tous les autres paramètres mesurés pour l'hématologie n'étaient pas significativement affectés par le temps d'accès et la durée de restriction. Le coût total de l'alimentation consommée était le plus élevé chez les

*Corresponding Author Email: obasa.oreoluwa@yahoo.com

lapins en croissance maintenus en 24 heures. Le coût de l'alimentation par kg de gain de poids n'avait pas été significativement influencé à travers les temps d'accès à la nourriture et les durées de restriction. Il a été conclu que pour un coût réduit de l'alimentation sans effet néfaste sur la performance et le profil hématologique, les lapins en croissance devraient être élevés sur un temps d'accès à l'alimentation d'au moins 4 heures pour une durée de restriction de 2 semaines.

Mots clés: Le temps d'accès à l'alimentation, la restriction alimentaire, l'hématologie et le coût.

Introduction

Interest in rabbit production in Nigeria has been on the increase in recent years. Rabbit occupies a unique niche in that it is a mini livestock that is easy to manage, highly prolific and has a short generation interval. The cost of feeding rabbits is however very high, a condition that also prevails for other livestock species in Nigeria (Adeyemi *et al.,* 2008). Currently, there has been an increased interest in studying feed restriction in rabbits as a means of reducing the cost of production (Adeyemi *et al.,* 2008). Growing rabbits usually have unlimited access to the feed and eat ad libitum. In a restricted feeding system, either the access of the animals to the feed is limited, or a fixed amount of feed is given. Feeding strategy in growing rabbits could be used to produce animals with maximum lean body mass, low feed conversion ratio, and best meat quality. The early life fast growth rate is accompanied by a number of problems, namely increased body fat deposition, high incidence of metabolic disorders, high mortality, and high incidence of skeletal diseases (Ebeid *et al.,* 2012). In the growing rabbits, an early feed restriction applied around post-weaning age could be of interest to improve feed efficiency (Yakubu *et al.,* 2007; Gidenne *et al.,* 2012), induce compensatory growth (Tůmová *et al.,* 2002; Foubert *et al.,* 2008), reduce carcass fat deposition (Tůmová *et al.,* 2004), improve digestibility of nutrients during the restricted feeding period (Tůmová *et al.,* 2004; Di Meo *et al.,* 2007).

Restricted rabbits are reported to have improved feed efficiency (Maertens and Peeters, 1988; Perrier and Ouhayoun, 1996). Improved digestibility of nutrients at restricted feeding period was found in rabbits by Ledin (1984a). Limiting feed intake depresses growth during the period of restriction, but reduced growth can be later compensated by realimentation. This phenomenon of accelerated growth following a period of feed restriction is termed "compensatory growth" (Tumova *et al.,* 2002). However, response to compensatory growth depends on the duration of feed restriction. Prolonged feed restriction diminishes the potential of compensatory growth (Leeson and Summers, 2005). In a bid to reduce the production cost without negatively compromising the performance and health status of the animals, this study was therefore aimed at identifying the time of access to feed and the restriction duration that will give the best growth performance, haematological profile and feed cost benefit.

Materials and Methods

Experimental site.

The experiment was carried out at the Directorate of University Farms of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The location lies within the rain forest vegetation zone of South West Nigeria with a mean rainfall of 1037 mm, a temperature of 34.7oC and a relative humidity of 82%.

Experimental rabbits and management.

A total of 60 unsexed rabbits of mixed breeds with weight ranging between 698 – 720 g were used for this experiment which lasted for 70 d. Prior to the arrival of the rabbits, the cages, drinkers and feeders were thoroughly cleaned and disinfected. At the beginning of the experiment the rabbits were randomly distributed into 12 treatment combinations with 5 replicates with 1 rabbit per replicate.

Daily routine management practices were carried out such as supply of clean water and feed, observing for sick rabbits, checking for mortalities and appropriate record keeping. In addition 100 g of freshly cut Tridax pucumbens were provided to each rabbit weekly.

Table I: Composition of the experimental diet

Ingrdient	%
Maize	47.50
Soyabeans meal	8.00
Wheat offal	31.00
Groundnut cake	10.00
Bone meal	3.00
Salt	0.25
Vitamins/ Premix*	0.25
Total	100.00
Determined analysis	
Dry matter (%)	89.45
Crude protein (%)	18.74
Crude fibre (%)	15.68
Ether extract (%)	4.48
Energy (KJ/kg)	10.93

**Composition per kg diet. Vitamin A. 4000000 IU, Vitamin D. 800000 IU, Vitamin E 40000mg, Vitamin K3 800mg Vitamin B1 1000mg Vitamin B2 6000mg, Vitamin B6 5000mg, Vitamin B12 25mg, Niacin 6000mg, Pantothenic acid 20000mg, Folic acid 200mg, Biotin 8mg, Manganese 300000mg, Iron 80000mg, Zinc 20000mg, Cobalt 80mg, Iodine 400mg, Selenium 40mg, Choline 800000mg.*

Experimental diet.

The composition of the diet is shown in Table I.

The diet was based on feed composition used for growing rabbits on the Directorate of University Farm of the Federal University of Agriculture Abeokuta, which was developed in line with the recommendations of Aduku (2004) and Merck (2011). The feed was given in mash form. Four levels of feed access time (24 h ad libitum, 6 h, 4 h and 2 h) and three restriction durations of 2, 4 and 6 wk were used.

Experimental design.

The experiment was arranged in a 4 x 3 factorial layout, consisting of four levels of feed access times (2, 4, 6 and 24 h) and three durations of restriction (2, 4 and 6 wk).

Data collection.

Growth performance Indices

The rabbits were weighed per replicate at the start of the experiment and subsequently on a weekly basis. Weight gain was taken by calculating the difference between the final body weight and previous body weight. Feed intake was recorded weekly for each treatment per replicate while left-over feed was subtracted from the total feed given to the rabbits in order to determine feed intake. Feed:gain ratio was calculated as feed intake per g of body weight gain. The total number of dead rabbits per treatment during the experimental trial was recorded and expressed as percentage (%) of total number of rabbits alive per treatment at the start of the experiment. Proximate analysis of the compounded ration was analyzed according to the method of AOAC (2005).

Evaluation of haematological parameters.

At the end of the study, 2.5ml of blood was collected from three rabbits per treatment. This was done through throat slitting into bottles containing ethylene diamine tetra acetate (EDTA) for haematological parameters (packed cell volume, hemoglobin concentration, white blood cell and red blood cell count) following standard procedure described by Davice and Lewis (1991).

Cost - benefit determination.

The prevailing market prices of the feed ingredients at the time of the experiment were used to estimate the unit cost of the experimental diet. Feed cost per kilogramme and cost per kilogramme of weight gain were calculated.

Statistical analysis.

All data collected from the 4 x 3 factorial design study were subject to a 2-way ANOVA analysis using SAS (2005). Significantly

($p<0.05$) different means were separated using Duncan's Multiple Range Test of the same statistical package.

Results and Discussion

Table II shows the main effect of feed access time and restriction duration on the performance of growing rabbits. Final weight, weight gain and feed intake was significantly ($p < 0.05$) influenced by the feed access time and the duration of restriction. Growing rabbits on 24-h and 6-h feed access time had higher ($p < 0.05$) final weight and weight gain. The favourable performance of growing rabbits on 6-h feed access time when compared with the ad libitum feeding (24-h feed access time) could be attributed to the restriction strategy which favoured proper compensatory growth. While the depression observed in the performance of the other restricted groups (2- and 4-h feed access time) could be attributed to the intensity or severity of the restriction which did not favour compensatory growth after the restriction period. Previous works by Yakubu et al. (2007), Gidenne et al. (2012) and Omoseibi et al. (2014) showed that the severity and length of feed restriction affected compensatory growth. It was also observed that the intensity of the duration of restriction affected the performance of growing rabbits.

From the present study, performance reduced as the duration of restriction increased. Total feed intake and daily feed intake at the end of the trial was significantly lower in restricted rabbit groups when compared to the ad libitum fed group. This present finding is in agreement with the reports of Perrier and Ouhayoun (1996) and Tumova *et al.,* (2003) who reported that feed intake in restricted fed rabbits was lower when compared to the ad libitum fed group. The feed:gain ratio was not significantly ($p>0.05$) influenced by the feed access time and restriction duration. This result supports the findings of Yakubu et al.(2007) who reported that no significant difference in feed:gain ratio between ad libitum fed and restricted fed rabbits. The similar values recorded in feed:

Table 2: Main effects of feed access time and duration of restriction on the performance of growing rabbits

	Feed access time (h)					Duration (wk)			
Parameters	**2**	**4**	**6**	**24**	**SEM**	**2**	**4**	**6**	**SEM**
Initial weight (g)	708.33	698.97	711.87	719.72	26.71	709.89	709.63	709.65	22.97
Final weight (g)	1622.72^{b}	1724.66^{ab}	1800.33^{a}	1855.36^{a}	47.72	1801.54^{a}	1766.5^{ab}	1684.27^{b}	44.19
Weight gain (g)	914.38^{b}	1025.69^{ab}	1088.46^{a}	1135.63^{a}	40.88	1091.65^{a}	1056.86^{ab}	974.61^{b}	40.21
Total feed intake (g)	3609.47^{c}	3953.73^{b}	4138.70^{b}	4652.69^{a}	118.93	4325.15^{a}	4016.20^{b}	3924.59^{b}	127.83
Daily weight gain (g/day)	13.06^{b}	14.65^{ab}	15.55^{a}	16.22^{a}	0.62	15.59^{a}	15.09^{ab}	13.92^{b}	0.57
Daily feed intake (g/day)	60.50^{c}	66.18^{b}	68.97^{b}	74.04^{a}	1.94	72.34^{a}	65.76^{b}	64.18^{b}	1.85
FCR[1]	4.03	3.95	3.88	4.14	0.16	4.07	4.05	3.88	0.14
Mortality (%)	7.14	13.33	0.00	0.00	5.12	5.00	5.26	5.00	5.08

^{a,b,c}Means in the same row not sharing common superscript are significantly different ($p<0.05$)

[1]FCR.: Feed conversion ratio

gain between the ad libitum fed groups and the restricted groups could be attributed to a better nutrient digestibility of the rabbits in the restricted groups. Perrier and Ouhayoun (1996) and Gidenne (1993) reported improved digestibility of nutrients and feed efficiency in rabbits at restricted feeding period. Feed access time and the duration of restriction had no influence on mortality percentage. This result is in consonance with the works of Osman (1991), Gidenne et al. (2003), Yakubu et al. (2007), Pinheiro et al. (2012) and Gidenne et al. (2010).

Table III shows the interaction between feed access time and duration of restriction. This study showed that the body weight gain of growing rabbits and feed intake were progressively reduced according to the intensity of the feed access time and duration of restriction. However, feed allocation regardless of the severity of the feed restriction still allowed for some growth and maintenance. It was observed that growing rabbits on 4-h feed access time for 2-wk restriction duration, 6-h feed access time for 2-wk duration and 6-h feed access time for 4-wk duration recorded similar performances and competed favourably with rabbits on 24- h feed access time (ad libitum). The performance recorded by rabbits in these groups in relation to the ad libitum fed group indicates that the intensity and duration of restriction they were exposed to could still permit a complete compensatory growth after the restriction phase. This result is in agreement with the work of Tumova et al. (2003) who reported a compensatory growth and increased weight gain in earlier restricted rabbits. However, the inability of the other restricted groups to catch-up with the ad libitum fed rabbits might be due to the intensity and duration of the restriction which did not allow them to compensate for the weight they lost during the restriction period. Studies have shown that the longer the period of under nutrition, the more difficult it is for rabbits to compensate for reduction in live weight (Yakubu *et al.,* 2007). The feed: gain ratio and the mortality were not influenced by the interaction between the feed access

Table 3: Interaction of feed access time and duration of restriction on the performance of growing rabbits

Feed access time	**2 h**			**4 h**			**6 h**			**24 h**			
Duration of restriction	**2 wk**	**4 wk**	**6 wk**	**2 wk**	**4 wk**	**6 wk**	**2 wk**	**4 wk**	**6 wk**	**2 wk**	**4 wk**	**6 wk**	**SEM**
Parameters													
Initial weight (g)	712.382	710.35	702.27	696.23	700.39	700.29	712.51	704.44	718.66	718.66	723.34	717.40	49.20
Final weight (g)	1696.2^{ab}	1696.0^{ab}	1476.0^{b}	1828.0^{a}	1668.0^{ab}	1678.0^{ab}	1822.0^{a}	1821.0^{a}	1698.0^{ab}	1885.10^{a}	1881.0^{a}	1860.0^{a}	74.36
Weight gain (g)	983.8^{ab}	985.7^{ab}	773.70^{b}	1131.8^{a}	967.6^{ab}	977.7^{ab}	1109.50^{a}	1176.6^{a}	979.3^{ab}	1141.6^{a}	1097.7^{a}	1167.7^{a}	69.55
TFI [1] (g)	4140.3^{abcd}	3605.7^{de}	3144.8^{e}	4375.10^{ab}	3661.3^{bd}	3877.6^{bcd}	4195.0^{ab}	4161.8^{abc}	4059.3^{bcd}	4652.7^{a}	4652.90^{a}	4652.80^{a}	183.70
ADWG[2] (g/ day)	14.05^{ab}	14.08^{ab}	11.05^{b}	16.16^{a}	13.82^{ab}	13.96^{ab}	15.85^{a}	16.80^{a}	13.99ab	16.30^{a}	15.68^{a}	16.68^{a}	0.95
Feed: gain ratio	4.25	3.78	4.07	4.03	3.84	3.97	3.84	3.62	4.19	4.08	4.28	4.06	0.24
Mortality (%)	20	0	0	0	20	20	0	0	0	0	0	0	5

^{abc} Means in the same row without common letter are significantly different at p<0.05

[1]TFI: Total feed intake

[2]ADWG: Average daily weight gain

Table 4: Main effect of feed access time and duration of restriction on the haematological parameters of growing rabbits

	Feed access time					Duration of restriction			
Parameters	**2 h**	**4 h**	**6 h**	**24 h**	**SEM[1]**	**2 wk**	**4 wk**	**6 wk**	**SEM[1]**
Packed cell volume (%)	35.88[b]	43.66[ab]	42.22[ab]	46.55[a]	2.29	43.72	38.66	42.22	2.59
Haemoglobin (g/dL)	11.56	13.55	13.52	14.73	0.63	13.63	12.60	13.51	0.71
Red blood Cell ($x10^2$)	5.52	5.88	5.97	6.47	0.30	6.16	5.46	6.07	0.29
White blood cell ($cumm^2x10^3$)	10.11	9.04	9.54	9.05	0.48	8.54[b]	9.50[ab]	10.20[a]	0.37
Lymphocytes	65.11	62.33	63.88	65.00	1.74	63.83	64.44	64.22	1.64
Neutrophils	33.66	36.66	34.44	33.33	1.63	34.55	34.00	35.00	1.57
Eosinophils	0.33	0.55	0.55	0.55	0.22	0.55	0.66	0.22	0.18
Basophils	0.55	0.33	0.44	0.55	0.30	0.55	0.44	0.33	0.23
Monocytes	0.33	0.33	0.66	0.55	0.27	0.61	0.44	0.22	0.23
N: L[2]	0.52	0.60	0.55	0.52	0.04	0.55	0.54	0.56	0.26

[abc]Means in the same row not sharing common superscript are significantly different ($p<0.05$)
[1]SEM: Standard error of mean
[2]Neutrophil lymphocyte ratio,

time and the restriction duration. This result is consistent with the works of Yakubu et al. (2007) and Lumturi et al. (2012) who reported no significant differences in the values obtained for feed: ratio and mortality.

Table IV shows the main effect of feed access time and duration of restriction on the haematological profile of growing rabbits. Feed access time had significant ($p<0.05$) effect on the values obtained for packed cell volume. These values increased significantly as the feed access time increased with rabbits on 2-h feed access time recording the lowest value (35.88%) while rabbits on 24-h feed access time obtained the highest value (46.55%). However these values were within the normal range recommended for growing rabbits by Tumova et al. (2007). According to Isaac et al. (2013) packed cell volume is involved in the transport of oxygen and absorbed nutrients. This implies that reducing the feed access time reduces significantly, the transportation of oxygen and absorbed nutrient in the blood. Furthermore, Chineke et al. (2006) reported that packed cell volume is significant in the diagnosis of anaemia and also serves as a useful index of the bone marrow capacity to produce red blood cells in mammals. Therefore, the downward trend observed in values obtained for the packed cell volume as the time access reduced could indicate that an anaemic condition is induced as the feed access time was reduced. This result agrees with the work of El–Monty (1991) who reported that feed restriction significantly reduced packed cell volume. The duration however, had no significant effect on the packed cell volume.

According to Isaac et al. (2013) red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al.,* 2013; Isaac *et al.,* 2013). According to the result of this present study, values obtained for red blood cell and haemoglobin were not significantly affected by the feed access time and the duration of restriction. However, values are within the range of red blood cell and haemoglobin for growing rabbits recommended by Tumova et al. (2007). This result is in consonance with the report of Ebeid et al. (2012) who reported no

Table V: Interaction of feed access time and duration of restriction on haematological parameters of growing rabbits

Feed access time	**2 h**			**4 h**			**6 h**			**24 h**			
Duration of restriction	**2 wk**	**4 wk**	**6 wk**	**2 wk**	**4 wk**	**6 wk**	**2 wk**	**4 wk**	**6 wk**	**2 wk**	**4 wk**	**6 wk**	**SEM**
Parameters													
Packed cell volume (%)	36.33	34.33	37.00	43.00	43.33	44.66	43.33	38.33	45.00	47.33	44.66	47.66	3.78
Haemoglobin (g/dL)	11.56	11.26	11.86	12.56	14.13	13.96	13.46	12.40	14.70	14.73	14.30	15.16	0.96
Red blood Cell ($x10^2$)	5.40	5.53	5.66	6.26	5.36	6.03	5.86	5.53	6.53	6.53	5.80	7.10	4.43
WBC[2] ($cumm^2x10^3$)	10.36^{ab}	8.93^{ab}	11.03^{a}	8.96^{ab}	8.60^{ab}	9.56^{ab}	10.53^{ab}	10.00^{ab}	8.10^{b}	8.50^{ab}	8.76^{ab}	10.40^{ab}	0.51
Lymphocyte	63.66	65.33	66.33	63.00	62.33	61.66	61.33	65.66	64.66	67.33	59.33	68.33	2.37
Neutrophil	35.00	33.33	32.66	36.33	35.66	38.00	36.00	33.0	34.33	31.66	39.00	29.33	1.89
Eosinophil	0.00	0.66	0.33	0.66	1.00	0.00	1.00	0.33	0.33	0.66	0.66	0.33	0.54
Basophil	0.66	0.33	0.66	0.66	0.00	0.33	0.33	1.00	0.00	0.00	1.00	0.66	0.025
Monocyte	0.66	0.33	0.00	0.00	1.00	0.00	1.33	0.00	0.66	0.33	0.00	1.33	0.24
[3]N:L	0.55	0.51	0.50	0.59	0.59	0.62	0.59	0.51	0.54	0.47	0.66	0.43	0.12

[abc]Means in the same row by factor with different superscript differ significantly ($p<0.05$)
[1]SEM: Standard error of mean
[2]WBC.: White blood cell
[3]N:L: Neutrophil lymphocyte ratio

significant difference in values for red blood cell and haemoglobin between rabbits fed ad libitum and those that had feed restriction. The duration of restriction had a significant effect on the white blood cell which interestingly increased as the duration increased. This result suggests an increase in disease resistance as duration increases or a need to increase the production of defensive mechanism that fights diseases as the duration increased.

Table V shows the interaction of feed access time and restriction duration on the haematological profile of growing rabbits. The interaction between feed access time and duration of restriction did not have significant ($p>0.05$) effect on the packed cell volume, haemoglobin, lymphocyte, neutrophil, eosinophil, basophil, neutrophil lymphocyte ratio. However, significant ($p<0.05$) difference was obtained for white blood cell count. All these values are within the range recommended by Ebeid et al. (2007). The non-significant difference observed in values for haemoglobin and red blood cell according to Isaac et al. (2013) indicates a normal transportation of oxygen and absorbed nutrient in the blood. The significant difference observed in the white blood cell with growing rabbits on the 2-h feed access time for 6-wk restriction duration recording the highest value implies that a more severe restriction strategy could increase the need for growing rabbits to produce defensive mechanism that fights diseases. The non-significant differences obtained for lymphocytes, neutrophils, eosinophils, basophils and monocyte imply that the interaction of feed access time and the duration of restriction did not adversely affect the production of antibodies.

Table VI shows the effect of feed access time and restriction duration on the cost – benefit ratio of growing rabbits. The total cost of feed per kg weight gain over the entire experimental period was not significantly influenced for both factors of feed access time and duration of restriction. However rabbits on the 6-h feed access time showed a better economic efficiency. The superiority of rabbits on the 6-h feed access time compared to the rabbits on 24-h feed access time arose from

Table VI: Effect of feed access time and duration of restriction on the cost – benefit ratio of raising growing rabbits

	Feed access time					Duration of restriction			
Parameters	**2 h**	**4 h**	**6 h**	**24 h**	**SEM**	**2 wk**	**4 wk**	**6 wk**	**SEM**
Total feed intake (g)	3609.47^{c}	3953.73^{b}	4138.70^{b}	4652.69^{a}	118.93	4325.15^{a}	4016.20^{b}	3924.59^{b}	127.83
Cost of feed /kg (N)	80.06	80.06	80.06	80.06	-	80.06	80.06	80.06	-
Total cost of feed consumed (Naira)	288.98^{c}	313.47^{bc}	331.34^{b}	372.49^{a}	8.98	346.271^{a}	321.54^{b}	314.20^{b}	10.05
Total cost of feed/ kg weight gain (Naira)	331.36	321.26	311.14	310.90	21.54	322.94	307.96	325.09	10.99

^{abc}Means in the same row by factor with different superscript differ significantly ($p<0.05$)
SEM: Standard error of Mean
abcMeans in the same row by factor with different superscript differ significantly ($p<0.05$)
SEM: Standard error of Mean

the fact that although, weight gain was not significant between the two treatments the amount required to feed rabbits on 6-h feed access time was however lower than the 24-hs feed access time. Maintaining rabbits on 6-h feed access time may therefore be a useful tool in saving feed cost and reducing the cost of production. This result corroborates the results of Adeyemi et al. (2013)) who reported better economic efficiency in rabbits restricted 80% of the ad libitum when compared with the ad libitum fed group.

Conclusion

From this study, it can be concluded that for a reduced body fat deposition and reduced cost of feeding without adversely affecting performance and blood profile, growing rabbits can be raised on not less than 4-hours feed access time for 2-weeks restriction duration.

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HYGIENE SURVEY FROM FARM TO MILK SUPPLY STAGE USING E. COLI ISOLATION AND ANTIMICROBIAL RESISTANCE TEST

Adem Hiko[1], Mezene Woyesa[2], Abdela Mohammed[2], Yimer Muktar[1]
[1]College of Veterinary Medicine, Haramaya University, Po. Box. 138 Dire Dawa, Ethiopia
[2]School of Veterinary Medicine, Collage of Medical and Health Sciences, Wollega University, Po. Box 395, Nekemte, Ethiopia

Abstract

Dairy industry involved various milking operation stages. Coliform and E. coli are marker and indicator of hygiene for fecal contamination of food at any point. Following 17 lactating dairy cow milking operations, five segments of stages consisting of 21 cut-off points were identified. Three hundred four (304) samples from various sources were collected and examined for E. coli to determine point(s) of milk contamination. Randomly selected isolates from all stages were exposed to chloramphenicol (C-30µg), neomycin (N-30µg), oxytetracycline (OT-30µg), polymyxin-B (PB-300IU) and trimethoprim-sulfamethoxazole (SXT-1.25/23.75/µg) susceptibility using disc diffusion techniques. E. coli was not isolated from currency used as control. An overall 43.2% E. coli with similar distribution at cow barn (35.3%), milking facility cleaning (38.8%), milking operations (34.8%) and product station (29.4%) but higher (87.2%) on milk marketing Ethiopian currency notes were observed. All of the 21 individual sampling points were almost equally E. coli positive and identified as equally important points. Out of 50 randomly selected and tested isolate for drug resistance, 2.0%, 4.0%, 8.0%, 46.0% and 74.0% were found resistant to polymyxin-B, chloramphenicol, trimethoprim-sulfamethoxazole, oxytetracycline and neomycin, respectively in ascending order. Single drug resistant were frequent (55.8%) followed by two drug resistant (36.6%) and MDR (9.7%).

Key words: Dairy; Milking stage; E. coli, Resistance, Haramaya University; Hygiene

*Author corresponding email: adex.2010ph@gmail.com

Introduction

Microbial contamination of milk and milk products can be generally occur from three main sources [from the udder, from the exterior of the udder, and from the surface of milk handling and storage equipment] (Pandey and Voskuil 2011; NDC 2011; Stephan and Kuhn 1999). Wrong methods of both hand and machine milking may also damage the teat or udder, allowing microorganisms easier access to the udder through the teat canal (Pandey and Voskuil 2011) with easily spreads of infection from one cow to the other during milking via contaminated milk, the hands of the milker, udder cloths and teat cup liners of milking machine. Infection may also occur during the interval between milking operations. Coliform and E. coli are often used as marker organisms for fecal contamination foods (Odonkor and Ampofo 2013). The Food and Agriculture Organization (2011) indicated fresh meat and raw milk are, nevertheless, considered as common vehicles for E. coli resulting in foodborne infections/intoxications (NDC 2011; Murinda et al. 2004). Murinda et al. (2004) reported 26.7% E. coli O157: H7 in dairy cows and/or their environment. Moreover, E. coli is one of causative agent of bovine mastitis (2.75%) (Stephan and Kuhn 1999). In Ethiopia, risk of E. coli was reported 8.1% from camel milk (Adugna et al. 2013), 17.0% from subclinical mastitis cases of small ruminate (Gebrewahid et al. 2012). Using E. coli, (Amenu et al. 2014) indicated association of the contaminated water used with quality of raw milk and traditionally processed milk products. Resistant E. coli strains to certain antimicrobial classes (Anupurba and Sen 2005; Hiko et al. 2008) from various food has been also reported worldwide (Hiko et al. 2008; Bell et al. 2002; El Khony et al. 2003). Study on milk contamination point identification with microbial from cow farm to milk supply stages were not yet conducted in Ethiopia. The aim of this study was to detect the possible points of milk contamination along dairy stage from cow farm to milk supply in Haramaya University Dairy Farm, Oromia-Ethiopia using E. coli examination with antimicrobial resistance test on the selected strains.

Materials and Methods

Ethical clearance: Study samples were also collected from dairy cows. Accordingly, the topic was presented to and approved by the College of Veterinary Medicine Ethical committee. Thus, international guidelines for animal welfare were followed under all circumstance.

Milking stages and operational activities of the studied dairy farm: The Haramaya University Dairy Farm was located in Eastern Hararghe Zone of Oromia Regional State, Ethiopia, at approximately 500 KMs East of Addis Ababa, the Capital City. The farm has routine milking operation, with different milking stages. Each of these stages has its respective milking operation activities. Thus, under the current condition, five different operational stages were identified in the farm and were described below:

- Dairy cow farm barn: prior to milking, lactating cows are kept in separate Lactating-cow's barn. Here, the cows are supplied with concentrate supplement diet.
- Cleaning operation stage: using tap-water and tap-water soaked towel, cleaning of cow's eats and different milking facilities including milk containers are practiced.
- Milking operation stage: this is a stage at which cow's are milked. This is practiced in milking barn using milking machine.
- Product and its supply stage: Milk product is collected in to a pooled container at milk collection location. After auditing, the raw milk product was transferred to public supply station.
- Milk-Ethiopia Currency exchange: From milk handler at public supply, the consumer can purchase using on-hand Ethiopian Birr currencies (Birr 1, Birr 5, Birr 10, Birr 50, and Birr 100 notes) exchange.

Sampling point identification:

Purposively sampling was done on 17 lactating cows kept in milking cow's barn prior

to milking from November 2014 to February 2015. Following dairy stage, stepwise sampling points were identified. Thus, sampling was done from lactating cow's farm barn up to milk supply station and from Ethiopian Birr (ETB) paper currency notes used for exchange at milk supply. Totally, 304 different samples from 21 various sampling points within the studied dairy stages (Table 1) that could have a possible contact with milk were collected.

Samples and sampling techniques:

Surface swabs, the milk, and the cleaning water samples were collected aseptically. Swabs from cow's farm barn, milking machine canal, teat cleaning towel, milking barn, milk collection facilities, cow teat and ETB were taken. Sample of milk from each teats was directly collected. Again, at collection and at supply, milk sample was taken from pooled milk. Water sample from pip-line were directly collected. Buffered Peptone Water (BPW) wet cotton swab was taken. Milk, water and swab samples were taken separately into sterile test tubes. Samples were transported to Microbiology Laboratory, College of Veterinary Medicine, Haramaya University, under chilled conditions using ice box. Microbiological examination was done within 24 h of collection.

Laboratory procedure

E.coli isolation and identification: Sample enrichment was done using BPW at 1: 10 proportion and overnight incubated at 37oC. After which a loop full was inoculated on to on Eosin Methylene Blue agar (Oxoid, UK). Characteristic metallic shine E. coli presumptive colonies were transferred to nutrient agar. Confirmatory biochemical test was done for Indole production, methyl red, vogues procures and citrate utilization (IMViC) according to Hirsh and Zee (1999) and Quinn et al. (2004).

Procedure for antimicrobial resistance test:

Antimicrobial resistance test was done according to Clinical Laboratory Standards Institute procedure (CLSI 2007; Bauer et al. 1966) using disc diffusion techniques. Fifty (50) selected strains from different sampling locations were sub-cultured on Nutrient agar (Merck, Germany) and incubated at 37oC for 24 hrs prior to testing. They were then inoculated into 5 ml of Brain Heart Infusion broth (BHI) (Merck, Germany) and again incubated for 16-24 hrs at 37oC. The inoculum density was standardized using a BaSO4 solution of a 0.5 McFarland standard (WHO 2011). Using sterile cotton swab deep into the broth culture, the inoculum were spread on Muller-Hinton agar (Oxoid, UK). Antimicrobial agents of chloramphenicol (C 30 µg), oxytetracycline (OT 30µg), trimethoprim-sulfamethoxazole (SXT 1.25/23.75µg), neomycin (N 30µg) and polymyxin B (PB 300U) (Oxoid, UK) were used. An interpretation was made based up on the zone of E. coli growth inhibition according to CLSI (2007) and Bauer et al. (1996). E. coli ATCC® 25922TM (USA) was used as control.

Data analysis:

The data obtained from investigation was entered in to Microsoft Excel 2007© and analyzed using IBM SPSS 20 and STATA 11. The significant of association for the prevalence of E. coli among explanatory variables at each points and stages were determined using percentage. Analysis of Variance (ANOVA) was used to calculate Chi-square test (□2) and 95% Positive Mid-exact confidence interval (CI) were used to determine the significant difference at $p < 0.05$.

Result

E. coli at dairy stage and sampling points: An overall 43.2% E. coli with similar distribution among dairy cow farm barn (35.3%), cleaning operations (38.8%), milking operations (34.8%) and milk product station (29.4%) ($p>0.05$) but significantly higher (87.2%; 95% CI, 73.2-94.3) than other stages on ETB currency notes used for milk exchange during supply ($p < 0.05$) were observed (Table 1).

Of 17 examined milking cows, three were with each one blind teats. All of the 21 identified sampling pointes from milking cow's barn up to milk at public supply station were E. coli positive with 25.0%-57.1%. It was 75%-100% on ETB currency notes used for marketing.

Table 1: E. coli the dairy stage and at individual sampling points within studied dairy farm stage

Dairy stage	Points**	Sampling points and sample types*	No. of Samples Examined	E. coli positive samples No. (%)	95 % E. coli Positive Mid CI ¶
Dairy cow farm barn	1	Milk-cow's barn swabs	17	6 (35.3)	17.3-59.0[A]
Cleaning operation	2	Towel swabs	8	2 (25.0)	7.5-60.0
	3	RF teat swabs□	16	5 (31.3)	14.2-55.9[a]
	4	RH teat swabs□	16	8 (50.0)	27.8-72.2
	5	LF teat swabs	17	6 (35.3)	17.3-59.0[a]
	6	LH teat swabs□	16	8 (50.0)	27.8-72.2
	7	Tap water	10	5 (50.0)	23.3-76.6
	8	Milk containers swabs	16	5 (31.3)	14.2-55.9[a]
		Sub total	**116**	**45 (38.8)**	**30.4-47.9[A]**
Milking operation	9	Milking barn swabs	14	8 (57.1)	32.3-78.7
	10	Milking machines canal swabs	36	13 (36.1)	22.4-52.5[a]
	11	RF teat milk □	16	5 (31.3)	14.2-55.9[a]
	12	RH teat milk □	16	5 (31.3)	14.2-55.9[a]
	13	LF teat milk	17	5 (29.4)	13.3-53.5[a]
	14	LH teat milk □	16	4 (25.0)	10.3-49.9[a]
		Sub total	**115**	**40 (34.8)**	**26.7-43.9[A]**
Product station	15	Milk at collection	9	3 (33.3)	12.2-65.2
	16	Milk at public supply	8	2 (25.0)	7.5-60.0
		Sub total	**17**	**5 (29.4)**	**13.3-53.5[A]**
Ethiopia currency (ETB) from milk handlers ***	17				
	18		8	8 (100)	66.4-100[b]
	19		10	10 (100)	71.5-100[b]
	20		8	6 (75.0)	39.9-92.5
	21		6	6 (100)	59.0-100
			7	4 (57.1)	24.5-84.3
		Sub total	**39**	**34(87.2)**	**73.2-94.3[B]**
		Grand total	**304**	**124 (43.2)**	**37.6-48.9**

**RF = Right font; RH = Right hind; LF = Left front; LH = Left hind*
□ *one teat was blind; ** the 21 sampling points; ***ETB = Ethiopia Birr*
¶ *= B is significantly higher ($p < 0.05$) than A; and b is significantly higher ($p < 0.05$) than a.*

Birr 1 and Birr 5 were 100% E. coli positive showing higher than most of other locations. Differences in E. coli prevalence among most of sampling pointes were not observed ($p > 0.05$). Within cleaning stage, E. coli was 50.0% in tap-water used for cleaning. With regards to milking operation stage, the prevalence of 57.1%, 36.1% and 25.0%-31.3% were observed in milking barn, milking machine and milk from teats, respectively with decreasing order but no significant difference. E. coli in milk at product collection (33.3%) and product (25.5%) were remains similar ($p > 0.05$).

Antimicrobial resistance profile the tested E. coli strains: Out of 124 isolated strains, 50 were randomly selected and tested for drug resistance. The strains showed 2.0%, 4.0%, 8.0%, 46.0% and 74.0% resistant to polymyxin-B, chloramphenicol, trimethoprim-sulfamethoxazole, oxytetracycline and neomycin, respectively in ascending orders (Fig. 1).

The distribution of resistant E. coli strain at individual sampling point within the studied dairy farm stage was shown in Table 2 bellow. Except the isolates from Right Front (RF) teat swab sampling point which showed no resistance, at least one or more of the tested strains showed resistant to one or more of drugs from the remaining points. One resistant strains to poymyxin-B was observed only at milk supply station (50%). Chloramphenicol resistant strains were observed from left front teat swab and right hind teat milk both at 50%. However, resistant E. coli strains to oxytetracycline and neomycin was frequently observed along most of the sampling points.

A total of 86.0% (43/50) isolates were found resistant for single to multiple drugs (MDR) of greater or equals to three. Single drug resistant were frequent (55.8%) followed by two drug resistant (36.6%) and MDR (9.7%). High single drug resistant for neomycin (75.0%), two drug resistant for oxytetracycline and neomycin (93.3%), and MDR for oxytetracycline, neomycin and trimethoprim-sulfamethoxazole (50.0%) were observed (Table 3).

Discussion

E. coli prevalence: The adherence of dairy farmers to strict food safety regulations, maintaining clean, safe facilities (Frye and Kilara 2008), sanitary environment and collecting milk

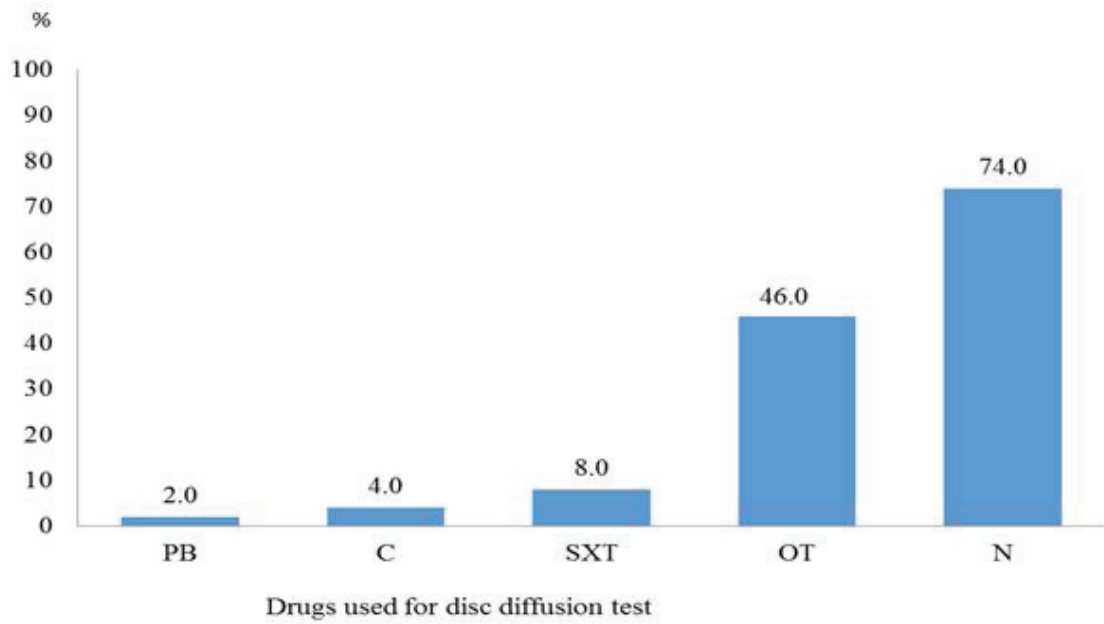


Figure 1: Drug resistance profiles of tested E. coli strain from dairy milking stage

Table 2: Antimicrobial resistance profiles of E. coli isolated from various sapling points along the studied dairy stage

Sampling points	No. of isolated E. coli	No. of tested E. coli	Resistant E. coli No. (%) for respective drug				
			PB	C	SXT	OT	N
Milk-cow's barn swabs	6	3	0	0	0	0	3 (100)
Towel swabs	2	2	0	0	0	2 (100)	1 (50.0)
RF teat swabs	5	3	0	0	0	0	0
RH teat swabs	8	4	0	0	0	1 (25.0)	4 (100)
LF teat swabs	6	3	0	1 (25.0)	1 (33.3)	2 (66.6)	2 (66.6)
LH teat swabs	8	4	0	0	2 (50.0)	3 (75.0)	3 (75.0)
Tap water	5	2	0	0	0	1 (50.0)	0
Milk containers swabs	5	3	0	0	0	1 (33.3)	2 (66.7)
Milking barn swabs	8	3	0	0	0	1 (33.3)	3 (100)
Milking machines swabs	13	3	0	0	0	1 (33.3)	3 (100)
RF teat milk	5	3	0	0	0	2 (66.7)	3 (100)
RH teat milk	5	4	0	1 (25.0)	0	0	4 (100)
LF teat milk	5	4	0	0	0	1 (25.0)	3 (75.0)
LH teat milk	4	3	0	0	1 (33.3)	2 (66.6)	2 (66.6)
Milk at collection	3	2	0	0		2 (100)	1 (50.0)
Milk at public supply	2	2	1 (50)	0	0	2 (100)	1 (50.0)
ETB note from milk handlers swabs	34	2	0	0	0	0	2 (100)
Total	124	50	1 (2.0)	2 (4.0)	4 (8.0)	23 (46.0)	37 (74.0)

**RF = Right font; RH = Right hind; LF = Left front; LH = Left hind*

Table 3: Single drug to MDR E. coli isolates from investigated dairy farm stages

Types of resistance	Total resistant No. (%)	Type of drug(s)	Resistant E. coli No. (%)
Single drug resistance	24 (55.8)	SXT	1 (4.5)
		OT	5 (22.7)
		N	18 (75.0)
		Sub total	24 (100)
Two drug resistance	15 (36.6)	C, N	1 (6.7)
		OT, N	14 (93.3)
		Sub total	15 (100)
MDR*	4 (9.7)	OT, N, PB	1 (25.0)
		OT, N, SXT	2 (50.0)
		C, OT, N, SXT	1 (25.5)
		Sub total	4 (100)
		Grand total	43 (100)

** MDR = multiple drug resistant*

in refrigerated holding tank until the processing plant (NDC 2011) for prevention of milk contamination were indicated. However, the presence of E. coli along all sampling points with almost absence of difference within the studied dairy farm stage is an indicator of the absence hygienic milking operation results in high possibility of milk contamination at any point along the stage. E. coli from teat surface indicates unhygienic conditions of udder resulting from inappropriate cleaning of udder before milking. In the intensive dairy farm, Pandey and Voskuil (2011) and NDC (2011) also showed prevalence of E. coli. The 43.2% overall E. coli at Haramaya University dairy farm with prevalence of 35.3% at dairy cow farm barn to 75.0% on milk exchange currency were similar with 35% report from Tunzia (Bali et al. 2013), 63% overall report from dairy and 45% from Khartoum-Sudan farms reported by Ali and Abdelgadir (2011). Again, E. coli at milking barn (57.1%) was similar with the reports of Ali and Abdelgadir (2011) from Khartoum North dairy farms (50%) and from Omdurman dairy farms (60%) Sudan but lower than the 11.3% from cow barn surfaces (Rahn et al. 1997) in UK. Tolle (1990) suggested, milk from healthy animal udder contains relatively few bacteria. But, 25.0%-31.1% of milk sample from cow teat were found E. coli positive in this finding indicating prevalence of mastitis in the farm. Presence of blind teat showing economic significance of the disease. Pandey and Voskuil (2011) and NDC (2011) also showed prevalence of E. coli and blind teats. Abunna et al. (2013) also reported 4.61% blind teats in dairy in Ethiopia. Present finding of E. coli from teats milk were higher than the 8.1% from camel mastitis (Adugna et al. 2013) and 17.0% from subclincal mastitis cases of small ruminate (Gebrewahid et al. 2012). Observing E. coli at equal rate on udder cleaning towel, milking machine, milking barn, teat swabs and milk container indicated unhygienic milking operation and those points are possible sources of milk contamination. Tolle (1990) and Standards (ANFS 2009) also indicated exterior of the udder and the adjacent areas, dairy utensils, milking machines, the hands of the milkers, from soil and dust as possible sources of milk contamination. Moreover, the 50% E. coli in water used for cleaning purpose indicated water as a possible sources of E. coli other pathogenies contamination of milking facilities, too which was also suggested by Eberhart (1997). Amenu et al. (2014) also indicated a positive correlation of E. coli counts in traditional milk and the water used during processing in Ethiopia. A 29.4% E. coli at in milk product station where 33.3% at collection and the 25% at public infection with hazards microbial including pathogenic E. coli strains. The finding were lower than the 80% each in milk from supply at Khartoum vending shops and Khartoum North vending shops (Ali and Abdelgadir, 2011) in Sudan. Australia New Zealand Food Standards (ANFS 2009) also indicated the possibility of milk contamination from already contaminated milking clusters contacting surfaces at postharvest environmental. Observation of E. coli on ETB notes from milk handler at supply was concomitant with microbial contamination of Ethiopian currency reported by Saripalli et al. (2014) and Girma et al. (2014) both from Ethiopia. This indicated contaminated currency also as possible source for postharvest product contamination.

Drug resistance:

The presence of drug resistant E. coli strain from present study could be associated frequent and under use of drug for the control and treatment of bacterial infectious diseases in the farm. The present 46.6% overall and 50% E. coli from cleaning water resistant to oxytetracycline were similar with report of Bahiru et al. (2013) to doxycycline and higher than the 10.6% resistant to tetracycline from from Zambian dairy cattle (Mainda et al. 2015), but lower than 81.3% resistant to tetracycline (Bahiru et al. 2013). The differance could be associated with geographical locations as well as in degrees of exposure to the drugs and genetics of E. coli strains. The 8.0% resistant to trimethoprim-sulfamethoxazole was similar with 4.49% reported by Mainda et al. (2015). Except for the 50% resistant strain

to oxytetracycline, resistance strain to other drugs was not observed in isolates from water, but Bahiru et al. (2013) reported for 12.5% resistant E.coli. This could be due to difference from water source where they tested isolate from hot waters in Ethiopia. The present 86.0% isolates found resistant to one or more drug were similar with the 87% resistant E. coli reported from dairy cattle (Sawant et al. 2007) indicating prevalence of resistant E. coli in the dairy farm. However, present finding was higher than the 42.5% reported by Sawant et al. 2007; Li et al. (2014) in E. coli from surface water. The fact that farm uses more antimicrobials, particularly to treat mastitis (Pandey and Voskuil, 2011) resulted in development of resistant strains. Mainda et al. (2015) also indicated cattle diseases mainly mastitis as the main drivers for using antibiotics on the different scale of dairy farms in Zambia. Significantly lower (9.7%) MDR E. coli than the 96.3% (Bahiru et al. 2013) from water, the 27.5% (Li et al. 2014) from surface water, the 22.6% (Hiko et al. 2008) from meat were observed in this study.

Conclusion

Presence of E. coli along all sampling points with the distribution of resistant strain almost along all the stages and at examined point's indicate the absence of good farming practices (GFP), good hygienic practices (GHP) and good management practices (GMP) application along studied dairy farm with the high possibility of milk contamination from various sources. There is a requirement for proper attention on the sanitary conditions from the farm to milk retail outlets to ensure milk quality and safety. Control of mastitis and milk quality, disinfection of the entire teats, use of WHO (2010) approved clean water, handling the exchanging currency by casher, hygienic milk handling at retail and consumer supply can reduce the risk. Regular Hazard Analysis of Critical Control Points (HACCP) implementation and drug resistance monitory in the farm will minimize contamination of milk, the production stage and resistant strain, too.

List of Abbreviations

CI = Confidence Interval; ETB = Ethiopian Birr; MDR = Multiple Drug Resistance; RF = Right font teat; RH = Right hind teat; LF = Left front teat; LH = Left hind teat.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

AH Hypothesis the idea, supervise the work, work in laboratory, analyze data and editing article; MW and YM work in laboratory, writing manuscript and editing; AM collect sample and work in the laboratory. All of authors assesses, read and approve the final version of the manuscript for submission.

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CONTAMINATION OF ETHIOPIAN PAPER CURRENCY NOTES FROM VARIOUS FOOD HANDLERS WITH E. COLI

Adem Hiko[1]*, Kasahun Abdata[1], Yimer Muktar[1], Mezene Woyesa[2], Abdela Mohammed[2]
[1]College of Veterinary Medicine, Haramaya University, Po. Box. 138 Dire Dawa, Ethiopia
[2]School of Veterinary Medicine, Collage of Medical and Health Sciences, Wollega University, Po. Box 395, Nekemte, Ethiopia

Abstract

Contamination rate of Ethiopian paper currency notes handled by various food handlers with E. coli and antimicrobial susceptibility was assessed. A total of 384 Ethiopian Birr (ETB) notes were randomly sampled from meat handlers at butchers, bread and the related food handlers at cafeteria, fruit and vegetables handlers at supermarket, and milk sellers both at open market and dairy station. Fifty control new currencies were also sampled from Commercial Bank of Ethiopia. Both surfaces of the currency were swabbed using wet sterile cotton and overnight incubated in buffered peptone water. A loop full was streaked on eosin methylene blue agar and followed by biochemical test on presumptive E. coli colonies. Randomly selected isolates were exposed to chloramphenicol (C-30µg), neomycin (N-30µg), oxytetracycline (OT-30µg), polymyxin-B (PB-300IU) and trimethoprim-sulfamethoxazole (SXT-1.25/23.75/µg) susceptibility using disc diffusion techniques. E. coli was not isolated from currency used as control. A total of 288 (75%) currency notes were found carrying E. coli. E. coli prevalence was ranges from 67.2% at open market milk sellers to 87.2% at dairy station milk sellers; from 64.8% on ETB 100 to 82.9% on ETB 1. Differences were not observed in E. coli prevalence on currency notes almost all food handlers ($p>0.05$). Susceptibility of tested isolates to each chloramphenicol, oxytetracycline and trimethoprim-sulfamethoxazole was 100%, and to polymyxin-B was 97.3%. High resistance (83.7%) was observed to neomycin. The finding indicates, contaminated food can be a source of E. coli for further contamination of currency which again transfer through various foods ready for consumption.

Key words: Antimicrobial, E. coli contamination, Food handlers, Public health, Haramaya, Paper currency

LA CONTAMINATION DES BILLETS DE BANQUE ÉTHIOPIENS PAR LA MANIPULATION DES ALIMENTS CONTENANT L'E. COLI.

Résumé

Le taux de contamination des billets de banque éthiopien manipulés par divers manipulateurs d'aliments contenant l'E. Coli et la sensibilité aux antimicrobiens a été évalué. Au total, 384 billets de Birr Ethiopien (BBE) avaient été sélectionnés de façon aléatoire parmi les vendeurs de viande dans les boucheries, vendeurs de pain, les serveurs dans les cafétérias, les vendeurs de fruits et légumes au supermarché et les vendeurs de lait à la fois au marché ouvert et à la laiterie. Cinquante nouvelles monnaies de contrôle ont également été échantillonnées auprès de la Banque commerciale d'Ethiopie. Les deux surfaces de la monnaie étaient essorées à l'aide du coton stérile humide et incubées pendant une nuit dans de l'eau peptone tamponnée. Une boucle pleine a été striée sur de l'agar bleu d'éosine méthylène et suivie d'un test biochimique sur des colonies présumées de l'E. Coli. Des isolats choisis aléatoirement étaient exposés à la susceptibilité du chloramphénicol (C-30µg), de la néomycine (N-30µg), de l'oxytétracycline (OT-30µg), de la poly myxine-B (PB-300IU) et du triméthoprime-sulfaméthoxazole (SXT-1.25 / 23.75 / µg) en utilisant des techniques de diffusion sur disques. L'E. Coli n'a pas été isolé de la monnaie utilisée comme témoin. On a retrouvé l'E. Coli sur 288 (75%) billets de banque. La prévalence de l'E. Coli variait de 67,2% à 87,2% respectivement chez les vendeurs de lait du marché ouvert et les vendeurs de lait à la laiterie; de 64,8%

*Corresponding author email: adex.2010ph@gmail.com

de BBE sur 100 à 82,9% de BBE sur 1. On n'a pas observé de différences dans la prévalence de l'E. Coli sur presque tous les billets de banque des manipulateurs d'aliments (p> 0,05). La susceptibilité des isolats testés à chaque chloramphénicol, oxytétracycline et triméthoprime-sulfaméthoxazole était de 100% et de 97,3% pour la poly myxine-B. Une résistance élevée (83,7%) était observée pour la néomycine. Les résultats indiquent que les aliments contaminés peuvent constituer une source de l'E. Coli pour une contamination accrue de la monnaie qui, une fois de plus, est transférée à travers divers aliments prêts à la consommation.

Mots-clés: Les antimicrobiens, la contamination par l'E. Coli, les manutentionnaires, la santé publique, l'Haramaya, le billet de banque.

Introduction

Currency notes are used for exchange of commodity and goods, including food. While so, it could be contaminated and represents as a vehicle for the transmission of pathogenic microorganisms in the environment and among humans (Michaels 2002; Xu et al. 2005). The Ethiopian currency called "Birr" is the second most used currency in Africa after Nigerian "Naira" for goods and services, including food and others, exchange in Ethiopia as in most other countries worldwide (Girma et al. 2014; Saripalli et al. 2014). The currency notes can be contaminated while counting using saliva, coughing and sneezing on hands followed by exchanging currency, placement or storage on dirty surfaces, poor hand washing post toilet. Such currency notes further act as a vehicle for bacteria to the next user (Barolia et al. 2011; Ngwai et al. 2014). Simultaneous handling of food and such contaminated currency could resulted in foodborne infection (Girma et al. 2014; Ngwai et al. 2014). Microbial contamination rate of currency handled by butchers (78.0 %) and food sellers (62.1 %) was also reported from Nepal (Lamichane et al. 2009). Microbial load of Bacilli, Coccus, Fungal species (Saripalli et al. 2014) from Axum-Ethiopia, Staphylococcus, Enterobacteriaceae, Micrococcus species, Streptococcus species (Girma et al. 2014) from Jimma-Ethiopia were reported from Ethiopian Birr. The Food and Agricultural Organization (2011) listed the possible means of food contamination and/or cross-contamination during growth and harvest (horticulture products), collection (milk) or slaughter (meat). Further contamination can occur during post-harvest handling, transport, processing and unhygienic food handling during preparation (FAO 2011). Escherichia coli (E. coli) was reported from currency at 5.6% from Ghanaian Currency Notes (Tagoe et al. 2009). However, E. coli carriage of Ethiopian currency notes handled by various types of food handlers was not yet assessed and the isolates were not also tested for drug susceptibility in and around Haramaya district. Thus, the aim of this study was to assess contamination rate of Ethiopian polymer currency notes handled by various types of food handlers with E. coli in and around Haramaya district with antimicrobial susceptibility test on the selected isolates.

Materials and Methods

Sampling:

A study was conducted at Haramaya district in east Hararghe Zone of Oromia Regional State, Ethiopia to determine the prevalence of E. coli on Ethiopia Birr (ETB) paper currency notes handled by various food type handlers with E. coli. Simple random sampling technique was applied both on the food handlers and the notes types of currency at Haramaya University (HU) and the surrounding, and at Haramaya town.

Sample sources:

The sample of currency was collected from meat seller at butcher, milk handler both at open market and dairy station, fruit and vegetables sellers' at supermarket, and bread and the related seller's at cafeteria. A random of Birr 1, Birr 5, Birr 10, Birr 50, and Birr 100 currency notes were collected from each notes. Age and sex of handlers were also considered. Using 95% confidence interval

(CI) with required 5% precision (Thrusfield 2005) sample size was calculated. A total of 384 ETB notes were sampled. During each sampling occasion, 10 each ETB currency notes were directly collected from Automatic Teller Machines (ATM) and Commercial Bank of Ethiopia branch in the study area for control purpose.

Sample collection:

All samples were collected aseptically by letting the selected individuals to drop a selected currency note into sterile polythene bags while substitution with equal currency. The polythene bags were promptly sealed, immediately labeled and transported to Microbiology Laboratory, College of Veterinary Medicine, Haramaya University (HU) for microbiological analysis.

Laboratory Procedures:

Both surfaces of each sampled currency were thoroughly swabbed aseptically using sterile Buffered Peptone Water (BPW) (Merck, Germany) soaked cotton on pre-sterilized aluminum foil. The swab was dipped into 10 ml BPW and incubated for 24 hr at 37oC. After which a loop full was streaked on Eosin Methylene Blue (EMB) agar (Oxoid, UK) and incubated overnight at 37oC. Characteristic metallic shine E. coli presumptive colony was transferred into Nutrient Agar for biochemical test using Indole production, Methylene red reduction, Voges Proskauer reaction and Citrate utilization (IMViC) tests according to Quinine et al. (2004). Escherichia coli ATCC® 25922™ (USA) was used as a control cultures. Antimicrobial susceptibility test was done on 43 randomly selected isolates by the Kirby-Bauer disk diffusion method (Bauer et al. 1966; CLSI 2007). The tested bacterium was taken from an overnight freshly grown culture and inoculated into 5ml Brain Heart Infusion Broth (Merck, Germany). The inoculated broth was incubated for 4 hours to approximately 106 CFU/ml at McFarland 0.5% level of turbidity (CLSI 2007). With this culture, a bacterial lawn was prepared on Mueller-Hinton agar (Oxoid, UK). Antimicrobials against (Oxoid, UK) including chloramphenicol (C 30µg), neomycin (N 30µg), oxytetracycline (OT 30µg), polymyxin-B (PB 300IU) and trimethoprim-sulfamethoxazole (SXT 1.25/23.75/µg). Result was interpreted using the diameter of zone of bacterial growth inhibition surrounding the disc (CLSI 2007).

Data Analysis:

The data obtained from laboratory examination was recorded in Microsoft excel 2007, analyzed using STATA 11 version and IBM SPSS 20. Percentage was calculated to evaluate for E. coli prevalence at all study variables. Significances of associated study factors/variables were compared using one way ANOVA. Significance of differences was considered at 95%confidence interval ($p < 0.05$) for study variables such as sample sources, currency note denomination and food types for E. coli positive.

Results

An overall 75.0% of ETB were found to contain E. coli. E. coli carriage rate of 73.8% and 77.1% were observed on currency from Haramaya University and the surrounding, and Haramaya town respectively. The contamination ranges from 67.2% at open market to 87.2% at dairy station milk sellers. With regards to currency notes, presence of E. coli ranged from 64.8% on the ETB 100 notes to 82.9% on ETB 1 notes. The prevalence of E. coli showed no any difference among and between all variables of study including sex and age categories food handlers (Table 1). However, no E. coli contamination was observed in control currency.

In particular, at Haramaya University and the surrounding, currency contamination was 73.8% with low (47.1%) at open market and higher (87.2%) at dairy station milk sellers showing significant difference ($p < 0.05$). However, among currency notes, age and sex, contamination ranged from 60.9% on ETB 100 to 85.4% on ETB 1 with no any differences ($p > 0.05$) within variables of study (Table 2).

With regards to Haramaya town, E. coli prevalence ranges from 72.5% on currency

Table 1: Rate of ETB contamination with E. coli among studied risk variables of food handlers.

Variables		No. of examined	Positive No. (%)	P-value
Location	HU and surrounding	240	177 (73.8)	P > 0.05
	Haramaya town	144	111 (77.1)	
Source–food type handled	Butcher—meat handlers	92	68 (73.9)	P > 0.05
	Open market— milk sellers	58	39 (67.2)	
	Dairy station—milk sellers	39	34 (87.2)	
	Cafeteria—Bread & relate handlers	92	70 (76.1)	
	Supermarket—Fruit & vegetables handlers	103	77 (74.8)	
Currency notes	Birr 1	82	68 (82.9)	P > 0.05
	Birr 5	81	61 (75.3)	
	Birr 10	77	60 (77.9)	
	Birr 50	73	53 (72.6)	
	Birr 100	71	46 (64.8)	
Handlers age in years	11 to 20	123	95 (77.2)	P > 0.05
	21 to 30	167	127 (76.1)	
	31 to 40	94	66 (70.2)	
Handlers sex	Female	192	143 (74.5)	P > 0.05
	Male	192	145 (75.5)	
Total		384	288 (75.0)	

Table 2: E. coli on ETB among studied risk variables of food handlers at Haramaya University and the surrounding.

Variables		No. of examined	Positive No. (%)	P-value
Source–food type handled	Butcher—meat handlers	62	44 (71.0)	P > 0.05
	Open market— milk sellers	17	8 (47.1)	
	Dairy station—milk sellers	39	34 (87.2)	
	Cafeteria—Bread & relate handlers	59	43 (72.9)	
	Supermarket—Fruit & vegetables handlers	63	48 (76.2)	
Currency notes	Birr 1	48	41 (85.4)	P > 0.05
	Birr 5	53	40 (75.5)	
	Birr 10	43	32 (75.5)	
	Birr 50	50	36 (72.0)	
	Birr 100	46	28 (60.9)	
Handlers age in years	11 to 20	81	67 (82.7)	P > 0.05
	21 to 30	94	67 (71.3)	
	31 to 40	65	43 (66.2)	

Variables		No. of examined	Positive No. (%)	P-value
Handlers sex	Female	114	84 (73.7)	P > 0.05
	Male	126	93 (73.8)	
Total		240	177 (73.8)	

Table 3: E. coli on ETB among studied risk variables of food handlers at Haramaya town.

Variables		No. of examined	Positive No. (%)	P-value
Source–food type handled	Butcher—meat handlers	30	24 (80.0)	P > 0.05
	Open market— milk sellers	41	31 (75.6)	
	Cafeteria—Bread & relate handlers	33	27 (81.8)	
	Supermarket—Fruit & vegetables handlers	40	29 (72.5)	
Currency notes	Birr 1	34	27 (79.4)	P > 0.05
	Birr 5	28	21 (75.0)	
	Birr 10	34	28 (82.4)	
	Birr 50	23	17 (73.9)	
	Birr 100	25	18 (72.1)	
Handlers age in years	11 to 20	42	28 (66.7)	P > 0.05
	21 to 30	73	60 (82.2)	
	31 to 40	29	23 (79.3)	
Handlers sex	Female	78	59 (75.6)	P > 0.05
	Male	66	52 (78.8)	
Total		144	111 (77.1)	

** Dairy station was not available*

obtained from fruit and vegetables handlers at supermarket to 81.8% on that of from bread and related food handlers at cafeteria. Here, the prevalence was ranges from 72.1% on ETB 100 to 82.4% on ETB 10 among currencies notes. Difference among and between factors/ variables within all variables of study were not observed ($p > 0.05$) (Table 3).

Susceptibility of selected and tested E. coli isolates from ETB notes to antimicrobial agents of under investigation was showed in the Table 4 below. It indicates that isolates from all variables was 100% susceptible to each of chloramphenicol, oxytetracycline and trimethoprim-sulfamethazole while there was frequent resistance to neomycin (83.7%) without double and multiple drug resistance strains.

Discussion

The present of E. coli on ETB at high level (75%), and handled by five food classes (meat handler, open market milk seller, dairy station milk seller, bread and related food handlers, and fruit and vegetable sellers) at different locations indicates the likelihood of public infection with harmful microbial including pathogenic E. coli strain in the studied area. It was higher than the 50% reported from Nigeria by Ngwai et al. (2011) in currency from samples including non-food items. This could be due to either contamination of the currency from food that had already contaminated with feces or others sources of E .coli. The moisture, sweat, air and others factors from the food at ambient temperature are driving force for

Table 4: Antimicrobial susceptibility/ profiles of contaminant E. coli from ETB

Variables		No. of E. coli tested	C No. (%)*	OT No. (%)*	SXT No. (%)*	PB No. (%)**		N No. (%)***		
			S	S	S	S	R	S	I	R
Food handlers and source	Meat handlers at butcher	8	8 (100)	8 (100)	8 (100)	7 (87.5)	1 (12.5)	-	2 (25)	6 (75)
	milk seller at open market	9	9 (100)	9 (100)	9 (100)	9 (100)	-	2 (22.2)	-	7 (77.8)
	milk seller at dairy station	2	2 (100)	2 (100)	2 (100)	2 (100)	-	-	-	2 (100)
	Bread & the related food handler at cafeteria	10	10 (100)	10 (100)	10 (100)	10 (100)	-	-	-	10 (100)
	Fruit & vegetables handlers at supermarket	14	14 (100)	14 (100)	14 (100)	14 (100)	-	-	3 (21.4)	11 (78.6)
Currency	Birr 1	9	9 (100)	9 (100)	9 (100)	9 (100)	-	-	-	9 (100)
	Birr 5	10	10 (100)	10 (100)	10 (100)	9 (90)	1 (10.0)	1 (10.0)	2 (20.0)	7 (70.0)
	Birr 10	9	9 (100)	9 (100)	9 (100)	9 (100)	-	1 (11.1)	-	8 (88.9)
	Birr 50	8	8 (100)	8 (100)	8 (100)	8 (100)	-	-	3 (37.5)	5 (62.5)
	Birr 100	7	7 (100)	7 (100)	7 (100)	7 (100)	-	-	-	7 (100)
Total		43	43 (100)	43 (100)	43 (100)	42 (97.7)	1 (2.3)	2 (4.7)	5 (11.6)	36 (83.7)

all are susceptible; ** intermediate was not observed; ***susceptible, intermediate and resistance was observed **S = susceptible I = intermediate **R** = resistance*

contamination and survival of E. coli on the currency with risk of further contamination of food at any point. Lamichane et al. (2009) and Saripalli et al. (2014) also provided similar suggestion. Jay et al. (2005) also indicated the possible risk of food cross contamination at any point. Moreover, the present finding was quite higher than the 1.3% E. coli species reported from the Polish Notes and Coins (Kalita et al. 2013) indicating the difference in hygienic handling condition between African and European country. Although the magnitudes were differ, microbial contamination of currency were reported globally (Kalita et al. 2013; Awel et al. 2010; Barolia et al. 2011; Girma et al. 2014). The absence of E. coli in new currency used for control but its presence on those circulating once indicates, the currency under usage harboring E. coli and other microbial. Similarly, Kalita et al. (2013) also indicated presence of opportunistic microbial on the used Polish Notes and Coins but not on the new ones. About greater than 70% of ETB were harboring E. coli and showed high and equal contamination within and among studies variables (location, food types, sources, ages and sex of handlers) of the currency in the studied district. However, the low E. coli rate on currency from open market (47.1%) than from dairy station (87.2%) at milk sellers (handlers) from Haramaya University and the surrounding indicated high prevalence of E. coli in dairy farm that can act as source of currency contamination through the milk. In Ethiopia, poor-currency-handling culture is widespread, and there is indiscriminate abuse of currency notes reported by Girma et al. (2014). Observation of E. coli on currency handled by vegetable handlers in the present study could also be contamination of the specific food from already contaminated dust, soil and water as well as from microflora of the body of handlers. Awel et al. (2010) and Barolia et al. (2011) also provided the same suggestion by tracing contaminant E. coli to dust, soil, water and the microflora of the body of handlers (hand, skin and others). Moreover, paper currency can acquire E. coli with droplets during coughing, sneezing, touching with contaminated hands or materials and from dirty surfaces at any point of placement. These was also shown by Ahmed et al. (2010) and Barolia et al. (2011). The Food and Agricultural Organization (2011) also suggested the persistence and growth ability of generic E. coli in many on foods. Thus, currency used for food marketing can acquire microbial including E. coli from already contaminated food items. Poor storage and handling, and frequent manual count activities also enhance currency cross contamination. Lamichane et al. (2009) and Sanjogita and Geeta (2014) provided similar suggestions.

With regards to specific ETB notes, E. coli harboring rate ranged from 60% to 85% showing high and similar exposure of most currency denominations. The relatively lower prevalence on Birr 100 and Birr 50 than other denomination (the Birr 1, Birr 5 and Birr 10) are similar with previously reported from Jimma (13.4% Enterobacteriaceae) by Girma et al. (2014) and from Axsum by Saripalli et al. (2014), both in Ethiopia. This could be due to frequent uses and high accesses of the latter notes which increase the risk of exposure for contamination. Diseases can spread through contact with fomites such as currency notes (Awe1 et al. 2010; El-Dars and Hassan 2005; Feglo and Nkansah 2010; Michaels 2002; Pope et al. 2002).

High susceptibility (97.7% to 100%) of the tested isolates to chloramphenicol, oxytetracycline, trimethoprim-sulfamethoxazole and polymyxin-B indicated the efficacy of these drugs for the treatments of E. coli infection. High and similar susceptibility of E. coli to most of these drugs were also reported by Hiko et al. (2008) and Kibret and Abera (2011) in Ethiopia. However, the significantly high (83.7%) resistance to neomycin could be due to frequent use the drug in medical sectors resulting for development of resistant strain that contaminate the currency from such personnel.

Conclusion

It was concluded that, the food which had already contaminated can be further act

as source of contamination of currency used for exchange purposes among various food handlers. Again, contaminated currency can act as serious source for likelihood of harmful microbial including pathogenic E. coli strains. Theses may leads to cross-transmission of ready-to-eat food items such as fruits, bread and the related with possibility of consumer infection. Thus, regular disinfection, use of banks, post offices and ATM with local languages, as well as application of ultraviolet light and fumigation, with parallel replacement of damaged and worn-out notes and education of food handlers were recommended to reduce contamination. The study also provides information on the likely choice of therapeutic antimicrobial agent for infections that might arise from this organism in the area. Further study on the prevalence of other microbial on currency including on coins at other part of the country were also recommended.

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Author's Contribution

AH propose the idea, supervise the work, analyze data and editing article; KA collect sample, data entry and writing manuscript; YM and MW writing manuscript and editing; and AM collect sample. All of investigators were work in laboratory, and assesses, read and approve the final version of the manuscript for submission.

Competing Interests

The authors declare that they have no competing interests.

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REPLACEMENT VALUE OF MILLET FOR MAIZE ON PERFORMANCE AND HAEMATOLOGICAL INDICES OF BROILER CHICKENS

Kafayat A[1], Bawa G S[2], Rano N B[1], Baba M[1]
[1]Department of Animal Science, Bayero University Kano
[2]Department of Animal Science, Ahmadu Bello University Zaria

Abstract

The experiment was conducted to determine the performance characteristics and haematological indices of broiler chickens fed varying levels of millet as a replacement for maize. Three hundred and thirty day old Marshal Broiler chickens were assigned to 5 dietary treatment groups. Each treatment was replicated 3 times with 22 birds per replicate in a completely randomized design. Five experimental diets were formulated with millet included at 0, 10, 20, 30 & 40% levels of inclusion. Weights of the birds were taken at the beginning and subsequently on weekly basis thereafter till the end of the experiment. Feed and water were fed ad libitum throughout the feeding trial which lasted for 56 days. On day 56, blood sample was collected from the wing vein of the birds using needle with syringe for haematological and blood biochemistry indices assay. The data collected were subjected to analysis of variance. Results indicated that birds fed 20% millet had better feed conversion ratio compared to those fed 40% millet. Inclusion of millet in broiler diets significantly reduced the water intake of birds. The total protein, globulin, albumin and glucose fell within the values recommended for normal chickens. It was concluded that millet can replace maize component in broilers diets up to 20% without any adverse effect on growth performance of broiler chickens.

Keywords: Performance, millet, broilers, haematological indices

LAVALEUR DE REMPLACEMENT DU MIL POUR LE MAÏS SUR LA PERFORMANCE ET LES INDICES HÉMATOLOGIQUES DES POULETS DE CHAIR.

Résumé

L'expérience a été menée pour déterminer les caractéristiques de performance et les indices hématologiques des poulets de chair nourris à des niveaux variables au mil en remplacement du maïs. Trois cent trente poussins de chair Marshal d'un jour ont été assignés à 5 traitements alimentaires. Chaque traitement était reproduit 3 fois avec 22 poulets par répétition dans un dispositif complètement aléatoire. Cinq régimes expérimentaux étaient élaborés avec du mil inclus à 0, 10, 20, 30 & 40%. Les poids des poulets étaient pris au début et ensuite sur une base hebdomadaire jusqu'à la fin de l'expérience. La nourriture et l'eau étaient servies à volonté tout au long de l'essai d'alimentation qui a duré 56 jours. Au jour 56, un échantillon de sang était prélevé de la veine de l'aile des poulets en utilisant une seringue à aiguille pour les analyses hématologiques et les indices de biochimie du sang. Les données recueillies étaient soumises à une analyse de variance. Les résultats ont indiqué que les oiseaux nourris à 20% de mil avaient un meilleur taux de conversion alimentaire que ceux nourris avec 40% de mil. L'inclusion du mil dans les aliments des poulets de chair avait considérablement réduit la consommation d'eau des poulets. La protéine totale, la globuline, l'albumine et le glucose sont tombés dans les valeurs recommandées pour les poulets normaux. Il a été conclu que le mil peut remplacer le maïs dans les aliments des poulets de chair jusqu'à 20% sans aucun effet négatif sur la croissance des poulets de chair.

Mots-clés: La performance, le millet, les poulets de chair, les indices hématologiques.

*Corresponding author email: bernardatohagan@yahoo.com

Introduction

Meeting the energy need of broiler birds in the semi-arid region has been a serious cause for concern due to high cost of energy and protein resources in the region. The combined effect of cost of production of maize and the intense competition for this grain between man and livestock especially in the drier areas of the tropics have made poultry rations to be expensive. This high cost and localized scarcity of commercial diets for poultry may, if not addressed, result in lower income to poultry farmers and reduced protein intake among the semi-arid communities (Clement *et al.,* 2010). One important measure that can be taken to alleviate such a threat is the use of alternative energy sources like millet and sorghum. The semi-arid conditions of West Africa with an ambient temperatures of > 38°C for most part of the year and low and poorly distributed rainfall make it more conducive for sorghum and millet production. (Clement *et al.,* 2010). The increase in price and non-availability of maize as energy source in poultry diets in the semi-arid region forces the poultry nutritionists to search for alternative energy sources for maize. Numerous studies were conducted in the past to explore the feasibility of incorporation of certain coarse cereals as energy sources in poultry diets (Kumar *et al.,* 1991; Rama Rao *et al.,* 2001). Among various cereals, millet and sorghum can be used as an alternative source of energy. NRC (1996) has reported that millet has no tannin, contains 5-7% oil and has higher protein and minerals than maize. Millets are a group of highly variable small-seeded grasses widely grown around the world as cereal crops or grains for fodder and human food. Millets are important crops in the semi-arid tropics of Asia and Africa (especially in India, Nigeria, and Niger), with 97% of millet production in developing countries (McDonough *et al.,* 2000). The crop is favoured due to its productivity and short growing season under dry and high temperature conditions.

Constraints to increase livestock production in the tropics after epidemic disease, is nutrition. The nutritional needs of farm animals with respect to energy, protein, minerals and vitamins have long been known, and these have been refined in recent decades. Production of maize has been on the decrease due to inadequate rainfall and loss of fertility in our soils. In addition, the demand for maize both by man and animal has been on the increase whereas the supply does not meet the demand. However, information on the feeding value of millet for poultry and its use in poultry diet would ease the pressure on maize, lower the feed cost and enhance poultry production. Moreover, due to high cost and scarcity of feed and feed ingredients poultry farmers still occasionally and haphazardly mix one or two ingredients together without due consideration for age and nutrient requirements of the class of birds involved. The objectives of the study are therefore to determine the performance characteristics and the haematological and blood biochemistry of broiler chickens fed varying dietary levels of millet.

Materials and methods

Experimental diets

The ingredients for the experimental diets were purchased from a reputable feed mill in Kano Nigeria. Five experimental diets with varying levels (0, 10, 20, 30 and 40%) of millet as a replacement for maize were formulated. The diets were designated as 1, 2, 3, 4, and 5 respectively. The gross composition of the experimental diets for broiler starter is presented in Table 1 for broiler while the composition of the broiler finisher was presented in Table 2.

The birds were fed the broiler starter diet from 0-4 weeks of age while broiler finisher from 5-8 weeks of age.

Birds and experimental design

Three hundred and thirty (330) day-old Marshal Broiler chickens purchased from Obasanjo farms a reputable distributor in Nigeria and were randomly allotted to the five dietary treatments in a Completely Randomized Design (CRD) (Steel and Torrie, 1980). The birds were reared in a deep litter house that

Table 1: Composition of the experimental diets (Broiler starter)

	Inclusion levels (%)				
Feed Ingredients	**0**	**10**	**20**	**30**	**40**
Maize	43.59	34.43	25.25	16.09	6.92
Millet	0.00	10.00	20.00	30.00	40.00
Soya bean meal	24.46	23.62	22.79	21.96	21.13
Groundnut cake	15.00	15.00	15.00	15.00	15.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Lime stone	4.00	4.00	4.00	4.00	4.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30
Vitamin premix*	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis (%) unless otherwise stated					
ME (Kcal/Kg DM)	2740.3	2577.42	2659.07	2496.45	2415.14
Crude protein	23.09	23.08	23.08	23.07	23.06
Crude fibre	4.06	3.99	4.02	3.95	3.91
Ether extract	3.8	3.7	3.8	3.6	3.5
Calcium	2.15	2.16	2.16	2.16	2.16
Phosphorus	0.28	0.31	0.30	0.33	0.35
Methionine	0.52	0.50	0.51	0.49	0.47
Lysine	1.74	1.70	1.72	1.68	1.66
Cost/ Kg diet (N)	77.00	78.00	79.00	81.00	82.00

**Vitamin premix (starter) contained (units/kg):Vitamin A, 1,000 I.U;Vit D, 2000 IU;Vit E, 5.0;Vit K, 2mg;Vit B1, 1.8 mg;Vit B2, 5.5 mg; Niacin, 27.5 mg; Pantothenic acid, 0.5mg;Vit B6 0.30 mg;Vit B12, 0.15mg; Folic acid, 0.75mg; Biotin, 0.6mg, Chlorine chloride, 300mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg; ME- Metabolizable Energy.*

was partitioned into pens as experimental units. Each treatment was replicated three times with 22 birds per replicate. Water and feed were offered ad libitum throughout the study period. The experiment lasted for eight weeks during which feed intake, live weight changes, feed conversion ratio and mortality rate were monitored. All the necessary routine management practices and the recommended vaccinations were strictly observed during the study period.

Birds and experimental design

Three hundred and thirty (330) day-old Marshal Broiler chickens purchased from Obasanjo farms a reputable distributor in Nigeria and were randomly allotted to the five dietary treatments in a Completely Randomized Design (CRD) (Steel and Torrie, 1980). The birds were reared in a deep litter house that was partitioned into pens as experimental units. Each treatment was replicated three times with 22 birds per replicate. Water and feed were offered ad libitum throughout the study period. The experiment lasted for eight weeks during which feed intake, live weight changes, feed conversion ratio and mortality rate were monitored. All the necessary routine

Table 2: Composition of the experimental diets (Broiler finisher)

	Inclusion levels (%)				
Feed Ingredients	**0**	**10**	**20**	**30**	**40**
Maize	48.40	38.87	29.42	19.42	10.54
Millet	0.00	10.00	20.00	30.00	40.00
Soya bean meal	12.65	12.18	11.63	11.63	10.51
Groundnut cake	17.00	17.00	17.00	17.00	17.00
Wheat offal	12.00	12.00	12.00	12.00	12.00
Lime stone	6.00	6.00	6.00	6.00	6.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30
Vitamin premix*	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis (%) unless otherwise stated					
ME (Kcal/Kg DM)	2676.81	2592.86	2509.49	2422.09	2342.91
Crude protein	19.36	19.49	19.59	19.89	19.77
Crude fibre	3.66	3.64	3.61	3.61	3.56
Ether extract	3.80	3.70	3.60	3.50	3.40
Calcium	3.17	3.17	3.17	3.17	3.17
Phosphorus	0.23	0.25	0.27	0.29	0.31
Methionine	0.47	0.46	0.45	0.44	0.43
Lysine	1.27	1.27	1.26	1.27	1.24
Cost/ Kg diet (N)	90.00	91.00	92.00	93.00	94.00

**Vitamin premix (starter) contained (units/kg):Vitamin A, 1,000 I.U;Vit D, 2000 IU;Vit E, 5.0;Vit K, 2mg;Vit B1, 1.8 mg;Vit B2, 5.5 mg; Niacin, 27.5 mg; Pantothenic acid, 0.5mg;Vit B6 0.30 mg;Vit B12, 0.15mg; Folic acid, 0.75mg; Biotin, 0.6mg, Chlorine chloride, 300mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg; ME- Metabolizable Energy.*

management practices and the recommended vaccinations were strictly observed during the study period.

Haematology and biochemical indices

At the end of feeding trial, 30 birds (6 birds per treatment) were selected and blood was collected from the wing vein using needle with syringe. Blood was drained into two different carefully labeled bottles for haematological and serum metabolite investigation. The blood samples for hematology were collected into bottles pretreated with an anti-coagulant Ethylene Diamine Tetra Acetic acid (EDTA). Blood samples for biochemical indices were collected into another sample bottles that does not contain EDTA. The parameters investigated include Urea, Sodium, Potassium, Creatinine, Glucose, Cholesterol, Triglyceride, ALP: Alkaline Phosphate, ALT: Alanine Amino Transferase, AST: Aspartate Amino Transferase, Total protein, Albumin, Globulin, Uric acid, Packed Cell Volume (PCV), Red Blood Cell count (RBC), White Blood Cell (WBC) and haemoglobin, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), Lymphocyte, Neutrophil, Monocyte, Eosinophil and Basophil (Jain, 1986).

Statistical analysis

Data collected on all parameters measured from the experiment were arranged and subjected to analysis of variance (ANOVA) using SAS software version 9.2 (2009).

Results

The performance of broilers fed the experimental diets is presented in Table 3. The final live weight and weight gain were not significantly (P>0.05) different across the dietary treatments. Feed intake of birds fed 20% millet was significantly lower compared to those on control, 10, 30, and 40% millet based diets. Feed conversion ratio for birds fed diets 1, 2, 3 and 4 were significantly better than those fed on diet 5.

Table 4 shows the blood chemistry of broilers fed varying levels of millet as a replacement for maize. There was no significant difference (P>0.05) in the Urea, Sodium, Potassium, Creatinine, Glucose, Cholesterol, Triglyceride, and ALP, Total protein, Albumin, Globulin and Uric acid levels in the blood across the treatments. However, there was significant difference (P<0.05) in the ALT and AST levels across the treatments where treatment 4 presented the highest level and treatment 5 had the lowest.

There was no significant difference (P>0.05) in the hematological indices measured amongst all the treatments (Table 5).

Table 3: Performance characteristics of broilers fed varying levels of millet as a replacement for maize.

	DIETS					
Parameters	**1**	**2**	**3**	**4**	**5**	**SEM**
Initial weight (g/b)	35.6[a]	34.8a	34.9[a]	34.9[a]	34.9[a]	1.13
Final weight (g/b)	1733.1	1595.7	1564.3	1632.7	1634.5	58.65
FI (g/b/d)	83.1[a]	79.3[a]	67.1[b]	78.1[a]	78.4[a]	2.80
WG (g/b/d)	28.3	27.6	25.6	27.6	27.1	0.95
FCR	2.9[a]	2.8[a]	2.7[a]	2.7[a]	3.17[b]	0.13
FC/gain (Naira)	242.2[b]	233.8[b]	230.9[b]	237.8[b]	278.7[a]	11.01
WI (ml/b/d)	293.5[b]	247.9[a]	249.5[a]	242.2[a]	245.8[a]	9.21

FI= Feed Intake, WG= Weight Gain, FCR= Feed Conversion Ratio, FC= Feed Cost, WI= Water Intake, SEM= Standard Error of Means. Means within the same row with different superscript are significantly different (P<0.05)

Table 3: Performance characteristics of broilers fed varying levels of millet as a replacement for maize.

	DIETS					
Parameters	**1**	**2**	**3**	**4**	**5**	**SEM**
Urea mmol/ L	3.53	3.12	3.21	3.60	3.50	0.26
Sodium mmol/L	136.50	135.67	136.67	138.67	142.33	2.05
Potassium mmol/L	6.37	5.98	6.1	6.27	6.07	0.27
Creatinine mmol/L	39.67	48.33	35.33	42.83	41.00	4.91
Glucose mmol/L	8.70	8.60	9.00	8.32	8.85	0.25
Cholesterol mmol/L	5.07	4.25	3.60	4.19	3.98	0.73
Triglyceride mmol/L	1.56	1.02	1.09	1.28	1.10	0.21
ALP U/L	206.00	212.83	210.33	210.33	224.00	9.52
ALT (SGPT) U/L	8.52ab	8.17ab	8.33ab	10.00a	5.67b	1.28
AST (SGOT) U/L	69.50bc	81.50ab	67.83bc	86.67a	56.33c	4.53

	DIETS					
Parameters	**1**	**2**	**3**	**4**	**5**	**SEM**
Total Protein g/L	38.00	40.00	38.67	45.33	42.00	3.92
Albumin g/L	14.50	13.83	14.00	15.67	14.50	1.00
Globulin g/L	23.50	26.17	24.67	29.67	27.50	4.32
Uric Acid	560.80	503.20	363.00	479.70	452.20	108.12

SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamic pyruvate transaminase. ALP= Alkaline Phosphate, ALT= Alanine Amino Transferase, AST= Aspartate Amino Transferase. SEM= Standard Error Mean, Means within the same row with different superscript are significantly different (P<0.05).

Table 5: Hematology indices of blood for broiler birds fed varying levels of millet as a replacement for maize

	DIETS					
Parameters	**1**	**2**	**3**	**4**	**5**	**SEM**
PCV	19.25	18.35	17.50	17.80	9.93	10.44
HB	10.48	7.90	9.50	6.03	5.03	5.08
RBC	1.39	1.77	1.21	1.55	0.74	0.86
WBC	63.10	52.05	58.90	52.50	21.93	30.91
PLT	61.67	73.83	51.17	85.67	57.00	46.91
MCH	50.65	36.87	52.27	26.03	22.67	24.71
MCV	92.78	72.60	96.07	77.07	44.70	48.14
MCHC	36.43	36.25	36.27	22.53	16.87	18.72
Lymphocyte	64.00	64.10	63.52	60.63	32.53	6.13
Neutrophil	1.28	2.42	1.73	1.20	0.47	0.95
MEB	1.38	3.40	1.42	4.83	0.33	1.89

PCV: Packed Cell Volume, RBC: Red Blood Cell count, WBC: White Blood Cell, HB: haemoglobin, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, Lymphocyte, Neutrophil, MEB: Monocyte, Eosinophil and Basophil. SEM= Standard Error Mean, Means within the same row with different superscript are significantly different (P<0.05).

Discussion

Final body weight, daily feed intake, daily weight gain (Table 3) showed no significant (P>0.05) differences among the treatment groups except for birds fed diet 3 whose feed intake differed significantly (P<0.05) from birds fed other dietary treatments. This was is in conformity with the findings of Andrew and Kumar (1992) and NRC (1996) who observed that millet can fully replace maize in chicken diets without adverse effects on feed efficiency and rate of gain. Similarly, Pour-Reza and Edriss (1997) stated that all dietary dietary maize portion of broiler diets can be replaced with low-tannin sorghum without adverse effects on live weight gain, feed intake and feed conversion ratio. Gualitieni and Rapoccinni (1990) and Jacob et al. (1996) have reported that low tannin sorghum has similar nutritional value with maize. Feed cost per weight gain was lower for birds fed 0, 10, 20 and 30% millet based diet compared to those fed 40% millet based diet. Considering the weight gain of the birds and the feed cost per weight gain, millet can completely replace maize in broiler chicken diets without adverse effects on performance. An added advantage is the reduced cost of feeding the broilers especially in areas where millet is highly cultivated.

The total protein, globulin, albumin and glucose falls within the values recommended

for normal chickens (Mittruka and Rawnsley, 1977 and Ibrahim, 2013).

The PCV level is low as compared with the levels reported by Ibrahim (2013) 28.4-35.7% and Martin et al. (1997) 21.3-30.8% for PCV. The Hemoglobin level also is low in birds fed diets 2, 4 and5 as compared with the findings of Ibrahim (2013) 9.5-11.7 g/dl for haemoglobin. However, the non-significant values of the haematology parameters across all dietary treatments, was probably an indication of adequate nutrition for these birds. In contrast to the foregoing, Ikhimioya et al. (2000) and Oladele et al. (2001) have linked lower values of such parameters to inadequate nutrition.

Conclusion

On the basis of the results of this study, it was concluded that millet can completely replace maize component in broilers diets up to 20% without any adverse effect on growth performance of broiler chickens.

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THE ECONOMIC VALUE OF GENETIC IMPROVEMENT OF THE ASHANTI BLACK PIG TO THE GHANAIAN PIG INDUSTRY

Hagan B A*, Asiedu P, Salifu S, Konlan S P
CSIR-Animal Research Institute, P. O. Box AH 20, Achimota, Accra, Ghana

Abstract

The aim of this study was to predict the trait and economic responses achievable for four economically important traits in the Ashanti Black pig (ABP) in Ghana using three different selection strategies. The objective traits modelled in this genetic improvement program were number of pigs weaned per sow per year (NW), back fat thickness (BFT), live weight at slaughter (SWT) and muscle depth (MD). All the traits modelled had favourable economic values with NW being the most important traits among the four. Generally, the genetic and economic returns from the multiple trait index selection (MTIS) and desired gains approach (DGA) were higher than those of the single trait selection strategy except for sole selection for NW (Ghana Cedis (GHS) 156.00). The economic returns for scenarios under MTIS and DGA were between 42 to 93% of the return from sole selection for NW. Single trait selection of MD, BFT and SWT gave the least economic responses. The three strategies modelled gave similar accuracy of selection ranging from 0.4834 to 0.6649 with scenarios under single trait selection giving relatively higher accuracies. The potential contribution of genetic improvement of ABP to the pig industry in Ghana is between GHS 1 060 000.00 and GHS 6 126 000.00. With clearly defined breeding objectives coupled with appropriate selection strategies, the value of the pig industry in Ghana could be improved with the ABP.

Keywords: Desired gains approach, Multiple trait index selection, Single trait selection, traits

LA VALEUR ÉCONOMIQUE DE L'AMÉLIORATION GÉNÉTIQUE DU PORC NOIR ASHANTI POUR L'INDUSTRIE PORCINE GHANÉENNE

Résumé

Le but de cette étude était de prédire le trait et les réponses économiques réalisables pour quatre traits économiquement importants dans le porc noir Ashanti (PNA) au Ghana en utilisant trois stratégies de sélection différentes. Les traits objectifs modélisés dans ce programme d'amélioration génétique étaient le nombre de porcs sevrés par truie par an (NPS), l'épaisseur de la graisse du dos (EGD), le poids vif à l'abattage (PVA) et la profondeur musculaire (PM). Tous les traits modélisés avaient des valeurs économiques favorables avec le NPS qui avait les traits les plus importants parmi les quatre. En général, les retours génétiques et économiques de la sélection de l'indice des traits multiples (SITM) et de l'approche des gains souhaités (AGS) étaient plus élevés que ceux de la stratégie de sélection des traits uniques sauf pour la sélection unique pour NPS (Ghana Cedis (GHS) 156,00). Les rendements économiques pour les scénarios de SITM et d'AGS se situaient entre 42 et 93% du rendement de la sélection de solde pour le NPS. La sélection d'un seul trait de PM, EGD et PVA a donné les réponses les moins économiques. Les trois stratégies modélisées avaient donné une précision similaire de sélection allant de 0,4834 à 0,6649 avec des scénarios sous la sélection de trait unique donnant des précisions relativement plus élevées. La contribution potentielle de l'amélioration génétique des PNA à l'industrie porcine au Ghana se situe entre 1 060 000,00 et 6 126 000,00 SGH. Avec des objectifs de sélection clairement définis couplés à des stratégies de sélection appropriées, la valeur de l'industrie porcine au Ghana pourrait être améliorée avec le PNA.

Mots-clés: L'approche des gains souhaités, la sélection d'indices de traits multiples, la sélection de traits uniques, les traits.

*Corresponding author e-mail: bernardatohagan@yahoo.com

Introduction

Pig production offers a very efficient and the fastest mode of providing the much-required animal protein for the Ghanaian population both in the short and long term (Rhule, Unpublished). The pig industry in Ghana is growing rapidly with an estimated growth in domestic pork production of 61% between 1999 and 2010 (SRID, 2011). An estimated 240 347 pigs were slaughtered for the domestic market in 2010 (SRID, 2011). The growth of the industry is due partly to the "Pork Show" fare organised by the Ministry of Food and Agriculture in 1988 to sensitise citizens about the significance of pig production and pork consumption as an important protein source (Awuku *et al.,* 1991). Prior to this fare, the popularity and consumption of pork was dwindling because of religious taboos and sanitation problems associated with pig production in Ghana.

The pig industry in the country is mainly characterised by smallholder farmers who keep between 5 to 20 animals in the household. There are however, few commercial farms in the industry keeping between 100 and 250 sows in their farms under intensive management system. The popular breed among smallholder farmers in Ghana is the Ashanti black pig (ABP).

The indigenous ABP is described by Devendra and Fuller (1979) and Barnes and Fleischer (1998) as having generally black skin with a small body size, a relatively long and narrow head with a prolonged snout. The ABP is an indigenous pig breed raised in most villages in Ghana (Baffour-Awuah *et al.,* 2005) under the traditional system of production (Ahunu *et al.,* 1995). It constitutes about 50% of the national pig population (Barnes, 1994). The relatively small body size of the ABP makes it easier to handle by both women and children. Holness et al. (2005) also described the indigenous pigs as better adapted to 'harsher' environments and poor management systems. They are more mobile and better equipped to scavenge. They are considerably less susceptible to heat stress and more resistant to most local diseases and parasites (Darfour-Oduro *et al.,* 2009). There is however wide variation with respect to economic important traits of ABP due to lack of selection in this breed and thus creating opportunities for genetic improvement.

The production systems of the pig industry in Ghana vary between semi-intensive to intensive system where animals are housed in mud-made structures with thatch roofing to cement-walled buildings with concrete floors and aluminium or zinc roofing. Feed fed to pigs ranges from conventional to non-conventional feed ingredients such as wheat bran, maize, 'pito' mash (brewer's spent grain), copra cake, cassava, cassava peels, yam peels, corn chaff, rice bran, cotton seed cake, soybean meal, kitchen waste, restaurant leftovers and hatchery wastes. Usually, the nutritional composition of the concentrate feeds is determined by the physiological age of the animal (weaner, grower or finisher). Finisher pigs are slaughtered at between 6-7 months of age when their live weights are 60 kg (Baffour-Awuah *et al.,* 2005). The average litter size at birth for ABPs is 5-7 with an average of 4 piglets weaned at 6 to 10 weeks of age.

In Ghana, the pricing of livestock is generally based on size and visual appraisal, however with the country achieving a middle-income status with its associated rise in disposable income and number of middle class citizens, meat quality is expected to gain importance among consumers. It is anticipated that back fat thickness (BFT) and muscle depth (MD) will play key role in consumer purchasing attitudes towards pork in the future (Pringle and Williams, 2000). The contribution of carcass traits to the overall profitability of the pig industry in Ghana is therefore apparent.

The main sources of breeding animals including pigs for smallholder farmers in the country are open market, farmers' own flock and from other farmers (Karbo and Bruce, 1997). This has partly led to high rates of inbreeding among their flock with its resultant inbreeding depression. It is one of the contributing factors to the low productivity of the ABP. There are no known stud breeders in the country who purposely breed improved boars for sale to

farmers. Similarly, the breeding objectives of ABP kept at Babile breeding station of Animal Production Directorate of the Ministry of Food and Agriculture is also not clear. It is therefore important for the nation to undertake a well-defined genetic improvement of this important breed to realise its full potentials.

The objectives of this study are to model and predict both trait and monetary responses that the nation stands to gain from genetic improvement of four economic important traits in the ABP using three different selection strategies.

Materials and Methods

Breeding objectives traits

The objective traits considered for this study were number of piglets weaned per sow per year (NW), live weight at slaughter at 210 days of age (SWT), carcass back fat thickness (BFT) and carcass muscle depth (MD). Number of piglets weaned per sow per year is the total number of piglets weaned by a sow in a year (Ek-Mex *et al.,* 2014). Under smallholder pig production systems, an ABP sow undergoes two parturitions per year with an average weaning age of 6-8 weeks. Live weight at slaughter is the average weight of a finisher pig at 210 days of age prior to slaughter (FAO, 2011). The BFT is the average thickness of fat at the back of a pig between the skin and the muscle tissues (Roongsitthichai and Tummaruk, 2014). Muscle depth is the depth of muscle tissues of finisher pigs' carcass at 210 days of age (Irie, 1992).

The true economic values of these objective traits were derived using the production model (Gibson and Dekkers, 2003). The economic value of a trait is defined as the increase in profit with a unit change in that trait while all other traits are constant. This represents the value of one unit of improvement of a trait. The constants used in deriving the economic values of the traits are given in Table 1. These constants are representative of mean trait values of unimproved population of ABP in Ghana.

The estimated economic values of the breeding objective traits are given in Table 2. Since mortality has been factored in the number of piglets weaned per sow per year, each piglet weaned per sow is therefore assumed to survive till it is sold as a finisher pig at a weight of 60 kg in 210 days of age with a BFT and MD

Table 1: Constants used in derivation of economic values of the objective traits and the income and expenses of the production system

Traits	**Averages/Base values**	
Number weaned per sow per year (NW)	12	
Back fat thickness (BFT), mm	22	
Live weight at slaughter (SWT), kg	60	
Muscle Depth (MD), mm	45	
Constants		
Days to slaughter, days	210	
Salvage age of sow, years	5	
Prices	**Costs, GHS**	**Income, GHS**
Pork price per kg at 22 mm BFT & 45 mm MD		10.00
Piglet cost per day	1.00	
Additional unit of MD		0.50
Additional unit of BFT in carcass	-0.50	
Cost of keeping sow per year	730.00	
Salvage value of sow		500.00

GHS – Ghana Cedis; GHS 3.70 = USD 1.00

Table 2: Derived economic values (in Ghana cedis, GHS) of the objective traits per finisher ABP

Traits	Economic value, GHS
Number of piglets weaned/sow/year	390.00
Back fat thickness	-30.00
Live weight at slaughter at 210 days	10.00
Muscle depth	30.00

Table 3 Heritability (h^2) and phenotypic standard deviation (σ^2_p) estimates of objective traits

Traitp	h^2	σ^2_P
Number of piglets weaned	0.10[a]	2.5
Back fat thickness	0.57[b]	2.9
Live weight at slaughter at 210 days	0.75[c]	9.7
Muscle depth	0.30[d]	2.68

[a]Van and Duc (1999); [b]Imboonta (2015); [c]Darfour-Oduro et al. (2009); [d]Mérour et al. (2009)

of 22 mm and 45 mm respectively. The negative value for BFT indicates that the value reduces with every unit increase in BFT beyond 22 mm.

Estimates of heritability (h^2) and phenotypic standard deviation (σ^2_p) of the objective traits are shown in Table 3. Estimates for the Ashanti black pig were not readily available hence the estimates from pigs of a fairly similar climate and management system were used. These parameters aided in predicting genetic change.

The phenotypic and genetic correlations of the objective traits are also shown in Table 4. These correlations are all assumptions with the help of information from pigs of different breeds and management system.

In this study, three different selection strategies were simulated. These were the multiple trait index selection (MTIS), single trait selection (STS) and desired gains approach (DGA). The traits used in the selection criteria were the same as the objective traits. Different sources of information were used in predicting breeding values used for parental selection. These information sources were similar for the different traits in the selection criteria except for NW. Table 5 gives the information sources and amount of records used per source.

Table 4: Phenotypic (above diagonal) and genetic (below diagonal) correlations among the objective traits (NW, BFT, SWT & MD)

	NW	BFT	SWT	MD
NW	1	-0.05	-0.14	0.01
BFT	-0.02	1	0.40	-0.06
SWT	-0.32	0.50	1	0.15
MD	0.02	-0.18	0.35	1

Table 5: Sources of information (Own, sire, dam, full sibs & half sibs) and amount of recording per source for each objective trait

Trait	Own	Sire	Dam	FS	HS
NW	1	0	1	5	10
BFT	0	1	1	10	20
SWT	0	1	1	5	10
MD	0	1	1	10	20

NW- Number of piglets weaned per sow per year; BFT-Back fat thickness; SWT-Live weight at slaughter 210 days; MD-Muscle depth; FS-Full sibs; HS-Half sibs

The total economic response for each selection strategy was calculated by the sum of the products of the trait's response or genetic gain and their economic values. The economic values (Table 2) served as the weights in the index.

Total economic response in GHS = EV_{NW}. R_{NW} + EV_{BFT}. R_{BFT} + EV_{SWT}. R_{SWT} + EV_{MD}. R_{MD} (Eq.1)

Where EV_x is the economic value of trait x

R_x is the response or genetic gain in trait x

The MTINDEX.XLS program (van der Werf, 2011) was used to simulate multiple trait index selection strategy whilst alternative scenarios were also examined. The alternative strategy included the single trait selection where only one trait was considered to be important for the profitability of the industry and the economic values of all other traits were assumed to be zero. In this strategy, the true economic values of the traits to be changed were used and all other traits assumed to have economic values of GHS 0.00, which were then inputted.

This study also simulated the desired gains approach. For this, the MTINDEX-_DESGAINS.XLS program (van der Werf, 2011) was used. The genetic gain or response of uninterested trait was set at 0.00001 and the right economic values for the other traits were used to attain the trait responses. However, the true economic values of all the traits were used in determining the total economic response as given in Equation 1.

The amounts of genetic change in the objective traits that can be achieved each year under the various strategies were also assessed. This was estimated by

$TER_x*(im + if)/(L_m + L_f)$ (Eq. 2)

Where TER_x = Total economic response for trait x

i_m = selection intensity for boars

i_f = selection intensity for gilts or sows

L_m = generation interval of boars

L_f = generation interval of gilts or sows

The GENUP program (Kinghorn, 1992) was used to estimate the selection intensities and generation intervals using the following assumptions:

1. 100 breeding sows and 5 boars with a mating ratio of 1:20
2. A weaning rate per sow was 0.8
3. Survivability of breeding animals was 90%
4. Age structure of the breeding animals is given below in Table 6.

The selection intensities of males and females from GENUP were im = 1.647 and if = 0.4619

Therefore (1.647 + 0.4619)/(2 + 3.37) which is 0.3927 was multiplied by the respective economic responses obtained under the various strategies to obtain the response per year.

The value of genetic improvement to the industry when genetic gains were disseminated to an estimated 100 000 pigs in the country was calculated for all the strategies.

Table 6: Age structure of the breeding pigs in the breeding programme

Age in years	1	2	3	4	5	Total	L
Males		5				5	2
Females		29	26	24	21	100	3.37

L-Generation interval

Results

The trait and total economic responses in GHS for the different selection strategies gave different outcomes. Single trait selection of NW gave the highest economic response of GHS 156.00 per generation whilst MTIS strategy and DGA of zero responses for BFT and SWT offered optimum responses of GHS 144.50, 145.00 and 144.60 respectively (Table 7). The responses of the objective traits were sensitive to their economic values. In the MTIS strategy, SWT that had a comparatively lower economic value of GHS 10.00 yielded a resultant unfavourable trait response of -0.97 per generation. On the other hand, NW, BFT and MD observed high and favourable responses due to their relatively high economic values.

Even though the single trait selection strategy gave suboptimal total economic responses except for NW, the respective trait responses for the selected traits were higher compared to those of MTIS and DGA (Table 7). The scenarios simulated under the DGA were that no genetic change was desired for one particular trait in the breeding objectives. All the scenarios stimulated under this approach out-performed those of STS strategy in total economic response except for NW. Single trait selection of NW yielded a total economic response of GHS 156.00 whilst the respective DGA yielded GHS 65.20.

Total economic responses per year under the different strategies are given in Table 8. The annual response per strategy showed a similar trend as those of the generational responses in Table 7.

The accuracy of selection is the ratio of standard deviation of index to that of breeding objective. The accuracies of all the strategies were moderate and ranged from 0.4701 to 0.6649 (Table 9). For the scenarios under the STS, accuracies were above 0.5 whilst those of DGA and MTIS were generally lower than 0.5 but for one scenario. The DGA and MTIS on average yielded higher standard deviation of breeding objective than those of the STS. The ranking of the strategies based on standard deviation of breeding objectives were generally similar to that of total economic responses.

The index weights of the sources of information used in the selection of animals for individual traits under MTIS are given in Table 10. The weights of the information sources are dependent on the amount of records, the economic values of the traits and the additive genetic relationships between the information source and the candidate for selection. The weights of all the information sources for BFT

Table 7: Economic values (EV) in Ghana cedis (GHS), trait responses (TR) in trait unit and total economic responses (TER) in GHS for multiple trait index selection (MTIS), single trait selection (STS) and desired gains approach (DGA) and scenarios under each strategy

Selection Strategy	EV				TR				TER (GHS)
	NW	BFT	SWT	MD	NW	BFT	SWT	MD	
MTIS	390.00	-30.00	10.00	30.00	0.35	-0.25	-0.97	0.34	144.50
Single Trait selection	390.00	0.00	0.00	0.00	0.40	-0.06	-2.50	-0.02	156.00
	0.00	-30.00	0.00	0.00	0.02	-1.44	-2.95	0.18	43.20
	0.00	0.00	10.00	0.00	-0.18	0.76	5.59	0.36	55.90
	0.00	0.00	0.00	30.00	-0.01	-0.29	2.23	0.90	27.00
Desire Gains Approach	60.24	-30.00	10.00	30.00	0.00	-0.44	2.83	0.79	65.20
	390.00	-12.46	10.00	30.00	0.36	0.00	-0.47	0.31	145.00
	390.00	-30.00	14.54	30.00	0.33	-0.12	0.00	0.41	144.60
	390.00	-30.00	10.00	-30.96	0.39	-0.15	-1.95	0.00	137.10

NW- Number of piglets weaned per sow per year; BFT-Back fat thickness; SWT-Live weight at slaughter 210 days; MD-Muscle depth

Table 8: Response per year in trait unit (number, mm, kg and mm for NW, BFT, SWT and MD respectively) and monetary unit per annum (GHS(R)) for the three selection strategies (Multiple Trait Index Selection (MTIS), Single trait selection and Desired Gains Approach (DGA), and the scenarios under each strategy

Selection strategy	NW	BFT	SWT	MD	GHS (R)
MTIS	0.14	-0.10	-0.38	0.13	56.75
Single trait selection	0.16	-0.02	-0.98	-0.01	61.26
	0.01	-0.57	-1.16	0.07	16.96
	-0.07	0.30	2.20	0.14	21.95
	-0.0039	-0.11	0.88	0.35	10.60
Desired Gains Approach	0.00	-0.17	1.11	0.31	25.60
	0.14	0.00	-0.18	0.12	56.94
	0.13	-0.05	0.00	0.16	56.78
	0.15	-0.06	-0.77	0.00	53.84

Table 9: Standard deviations (SD) of selection index and breeding objective and accuracy of the three selection strategies (MTIS, Single Trait Selection and Desired Gains Approach)

Selection strategy	SD of Index	SD of breeding objective	Accuracy
MTIS	146.04	302.10	0.4834
Single Trait selection	154.44	308.32	0.5009
	43.25	65.68	0.6585
	55.86	84.00	0.6649
	27.00	44.04	0.6131
Desired Gains Approach	65.36	106.49	0.6138
	143.84	299.73	0.4799
	143.83	300.45	0.4787
	135.35	287.94	0.4701

MTIS-Multiple Trait Index Selection

Table 10: Index weights of the various sources of information under multiple trait index selection (MTIS) strategy for Number weaned per sow per year (NW), Back fat thickness (BFT), Live weight at slaughter (SWT) and Muscle depth (MD)

Trait	Own	Dam	Sire	Full-sib	Half-sib
NW	30.669	13.047	-	58.651	53.821
BFT	-	-0.916	-0.418	-4.799	-1.14
SWT	-	-0.462	-0.782	-0.020	0.451
MD	-	4.737	2.269	28.955	16.850

are negative since the economic value for this trait is negative. No information was taken on progeny for any of the traits hence any index weight for this source.

Table 11 compares the annual value of genetic improvement to the pig industry from the various strategies when gains are disseminated to 100 000 pigs.

Table 11: Expected value addition of genetic improvement to the pig industry from the various strategies

Strategy	Value of Gain to industry, GHS
Multiple trait index selection	5 675 000.00
Single trait selection	6 126 000.00
	1 696 000.00
	2 195 000.00
	1 060 000.00
Desired Gains Approach	2 560 000.00
	5 694 000.00
	5 678 000.00
	5 284 000.00

Discussion

The drivers of profitability of most livestock production systems depend not only on a single but many traits. In genetic improvement programmes for livestock in the tropics therefore, several traits could be improved altogether provided they possess some economic values. This will ensure efficient use of scarce resources.

Among all the strategies and scenarios modelled in this study, single trait selection of NW gave the highest economic return of GHS 156.00 per generation and GHS 61.26 per annum. Though NW has a low heritability, its high economic value coupled with information from own source in estimating breeding values led to the overall increase in the value of the progress for this scenario. It is therefore possible to improve only NW in the ABP in situations where there are limited resources for the genetic improvement of several traits.

The individual trait responses for each objective trait under scenarios in the STS were higher than the corresponding traits responses in MTIS i.e. 0.40 vs 0.35, -1.44 vs -0.25, 5.59 vs -0.97 and 0.90 vs 0.34 for NW, BFT, SWT and MD respectively. This is expected as the genetic change in each individual trait decreases with an increase in the number of selection traits (Stewart *et al.,* 1999). However, the overall economic responses per generation for STS were the least expect for NW.

Multiple trait index selection and DGA of zero responses for BFT, SWT and MD also yielded high economic returns of between GHS 137.10 and GHS 145.00 per generation. The expected response of GHS 144.50 for MTIS agrees with Simm (2000) that MTIS is the most efficient method of improving several traits at the same time. Though MTIS did not give the highest economic return, it was impressive considering that all the objective traits were being improved jointly in this strategy. Multiple trait index selection combines the true economic values of all the traits in the breeding objective as weights in the selection index for animals. There were favourable trait responses of 0.35, -0.25 mm and 0.34 mm per generation for NW, BFT and MD respectively under MTIS with an unfavourable response of -0.97 kg for SWT. This could be explained by the fact that SWT had the least economic value in the index hence minimal emphasis was placed on it for improvement relative to the other traits. The negative correlation between NW and SWT could also partly be attributed to the observation considering the wide difference between their economic values. Multiple trait index selection seeks to optimise total response and not just trait response and so will do this taking into consideration the weights of the traits in the index. Should the economic value of SWT increase in the future due to changes in market forces, the trait response will also increase. For instance, when the economic value of SWT is doubled (GHS 20.00) with all other parameters remaining the same, the

response for this trait will improve significantly to 1.16 kg with MD also increasing to 0.47 mm whilst NW and BFT will reduce to 0.28 and 0.04mm respectively. However, the overall economic return increased marginally to GHS 145.30. The overall economic responses as well as response per trait are indeed dependant on the economic values of the traits (Kadarmideen and Simm, 2002). The increase in response of SWT with a corresponding increase in MD is due to the positive genetic and phenotypic correlations between these two traits. Traits with unfavourable correlations are difficult to improve jointly hence the reduction in response for NW and BFT as SWT increases with an increase in its economic weight. Even though trait responses per generation and per annum under MTIS were not the highest, the total economic response of the strategy was among the highest. This strategy therefore maximises the change in breeding values for total economic merit.

The value of genetic improvement to the industry under MTIS was a colossal GHS 5,675,000.00 per annum. It should however, be noted that in reality this value might be slightly lower considering the generation gap between the nucleus population of breeding animals and the commercial population.

Single trait selection strategy assumes that only one trait is important for the profitability of the livestock industry. This strategy yielded the maximum response for the trait on which selection was done with suboptimum responses for other traits in the breeding objectives. This is because in STS, all emphasis was placed on only one trait leading to a high response in that trait. However, because correlations exist among traits, there will be responses in all other traits for which emphasis were not placed on for genetic improvement. For instance, selection on only NW yielded a response of 0.40 with correlated responses of -0.06 mm, -2.50 kg and -0.02 mm for BFT, SWT and MD respectively. Though the true economic weights of the traits (BFT, SWT and MD) were not used, the overall economic response of GHS 156.00 was the highest. This is due strongly to the very high economic value of NW. However, the other scenarios in the STS ranked the least in economic responses. It is therefore important to include all the economic values of traits that contribute to profitability in the selection criteria of a breeding program to obtain optimum merit in the improvement programme.

The third strategy, DGA, yielded quite good overall economic returns. In this approach, a predetermined response is set for a trait and the economic weights needed to achieve this desired response are estimated. For instance, when a zero response was desired for NW, the economic value needed to attain that was GHS 60.24. This obviously was lower than the true economic value of the trait hence a lower overall economic return of GHS 65.20 per generation and GHS 25.60 per annum. In this particular scenario, the low heritability of NW coupled with the drastic reduction in its economic value has greatly reduced the overall economic return expected. Simm (2000) indicated that DGA is suboptimal because it puts too much emphasis on traits that are difficult to improve because they have low heritability. For the other scenarios under DGA however, the overall economic responses were similar to that of MTIS. This could be due to the relatively high heritability of the traits and slight changes in their new economic values. In this study, the DGA ranked among the highest in terms of overall economic returns. The choice of this strategy for genetic improvement is inevitable if knowledge of economic value of a trait is not accurate or available. In such a situation, an idea about the direction in which we want to change the trait might be useful.

The accuracy of the selection criteria is dependent on the sources and amount of information used in selection. The higher the amount of information used in estimating the breeding value of an animal for selection, the higher the accuracy of the selection index. In this study, the sources of information used in selection were the same for all the selection strategies hence the seeming similarities in the accuracies. The use of additional information from progeny in the index will increase the accuracy and subsequently the response to

selection. This is because accuracy is directly related to response to selection (Simm, 2000). For instance, if 10 progeny records on each trait were included in the index, index standard deviation and accuracy of MTIS would increase to 182.71 and 0.6048 respectively whilst the standard deviation of the breeding objective will remain the same (302.10). The total economic return would also increase from GHS 144.50 to GHS 173.80 per generation. The use of more information would increase the accuracy of selection index and response to selection but not the standard deviation of breeding objectives. The cost of additional information should always be compared with the gains achievable to determine whether it is worthwhile including that information. The use of information on progeny in selection will also increase the generation interval hence a reduction in the annual genetic gain (Dickerson and Hazel, 1942; Lillehammer *et al.,* 2011). The rate of inbreeding may also increase from the use of relative information in selection since superior animals are more likely to be related to each other.

Accuracy of selection is also a function of heritability. Generally, the scenarios under STS had higher accuracies with sole selection for SWT being the highest. Under STS, the accuracies of selection ranked similarly with the heritabilities of the traits.

The index weights of the sources of information are dependent on the amount of records on each information source and the additive genetic relationship between the source and the candidate animal. Under smallholder systems in Ghana, it is difficult to measure BFT, SWT and MD on candidate animals without sacrificing them due to the absence of appropriate equipment and financial constrains. Information on relatives was therefore used in selecting for such traits. The inclusion of genomic information in the selection of animals will also increase the accuracy significantly without increasing generation interval. This will in effect greatly increase response to selection (Lillehammer *et al.,* 2011).

Genetic improvement brings about genetic merit and this has an implication on economic returns to the industry. The benefits of genetic improvement of the ABP to the pig industry in Ghana assuming the genetic gains achieved were disseminated to 100, 000 would range from GHS 1 060 000.00 to GHS 6 126 000.00. This is quite impressive for a relatively young industry when compared to older industries like the ruminant and poultry. It should however, be noted that the existence of a generation gap between nucleus and commercial flock might hinder the full realisation of this economic potential. However, advantage could be taken of the high reproductive rates of pigs in general to achieve the benefits of genetic improvement in the industry in Ghana.

Conclusion

The profitability of the pig industry is not limited to just one but several traits. Any genetic improvement program for the ABP therefore calls for the inclusion of all traits with economic values in the breeding objectives. All the three selection strategies modelled in this study showed positive economic returns. Besides STS of NW, which gave the highest economic response, MTIS and DGA also gave high economic returns. The value of the pig industry in Ghana can be improved when careful attention is given to the definition of the breeding objectives and the use of appropriate selection strategies. Adequate attention should also be given to the estimation of genetic parameters of the ABP in Ghana to enhance the success of any breeding program for this breed.

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Conflict of interest

The authors declare that they have no competing interests.

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EFFECT OF CONCENTRATIONS OF ARTEMIA SALINA ON ZOOTECHNICAL PERFORMANCES OF FARFANTEPENAEUS NOTIALIS IN LARVAL STAGES.

Guegang Tekou[1]*, Claudine Tekounegning Tiogué[2], Paul Zango[1], Minette Eyango Tomedi[1, 2]
[1]Institute of Fisheries and Aquatic Sciences at Yabassi, the University of Douala, P.O.Box 2701, Douala, Cameroon. email : Tomedi_tabi@yahoo.fr, paul1zango@hotmail.com
[2]Laboratory of Applied Ichthyology and Hydrobiology, Department of Animal Productions, Faculty of Agronomy and Agricultural Sciences, the University of Dschang, PO. BOX 222 Cameroon. email : tekou_claudine@yahoo.fr

Abstract

Effect of concentrations of living food Artemia salina on survival and growth performances of marine shrimp Farfantepenaeus notialis (Pérez Farfante, 1967) from the Zoe 3 to Post larvae 1 stages, was carried out at AquaSol (Aquaculture Solidarité) structure in IRAD (Institute of Agricultural research for Development) of Kribi in Cameroon between July and August 2013. 3600 larvae (Zoe 3) born from a pregnant female (with total weight 70.6 g and 20 cm total length), collected in the natural environment by a fisherman using a bottom thread were used. The larvae were bred in an experimental device consisting of 6 treatments seeded in 3 replicates each. Treatments were randomly distributed into 18 cylinder-conical plastic bottles. Larvae were fed at different levels of concentration of imported Artemia salina nauplii (AN) (0 AN/ml, 1 AN/ml, 2 AN/ml, 3 AN/ml, 4 AN/ml and 5 NA/ml), Microalgae Thalassiossira pseudonana cultivated in the station and with a dry imported food. The best performances of survival, growth and metamorphosis were produced by 3 AN/ml concentrations of Artemia. The cost of food has increased with the concentration level of Artemia. Then, the production of viable shrimp post larvae and cheaper may be done at 3 AN/ml optimal concentrations of Artemia.

Key words: Biotic factors, Farfantepenaeus notialis, growth, survival, Cameroon

L'EFFET DES CONCENTRATIONS D 'ARTÉMIA SALINA SUR LES PERFORMANCES ZOOTECHNIQUES DE FARFANTEPENAEUS NOTIALIS AUX STADES LARVAIRES.

Résumé

L'effet des concentrations de nourriture vivante Artémia salina sur les performances de survie et de croissance des crevettes marines Farfantepenaeus notialis (Pérez Farfante, 1967) des stades Zoe 3 au stade post larves 1, a été effectuée entre juillet et août 2013 à la structure Aqua Sol (Aquaculture Solidarité) à IRAD (l'Institut de la Recherche Agricole pour le Développement) de Kribi au Cameroun. 3600 larves (Zoe 3) nées d'une femelle enceinte (avec un poids total de 70,6 g et une longueur totale 20 cm), recueillies dans l'environnement naturel par un pêcheur utilisant un fil inférieur ont été utilisées. Les larves étaient élevées dans un dispositif expérimental consistant en 6 traitements ensemencés en 3 répétitions chacune. Les traitements ont été distribués de façon aléatoire dans 18 bouteilles en plastique cylindriques coniques. Les larves étaient nourries à différents niveaux de concentration des nalpus d'Artémia salina (AN) (0 AN / ml, 1 AN / ml, 2 AN / ml, 3 AN / ml, 4 AN / ml et 5 NA / ml), Microalgae Thalassiossira Pseudo nana cultivé dans la station et avec une nourriture sèche importée. Les meilleures performances de survie, de croissance et de métamorphose étaient produites par les concentrations de 3 AN / ml d'Artémia. Le coût des aliments avait augmenté avec le niveau de concentration d'Artémia. Ensuite, la production de larves de crevettes viables et moins coûteuses peut être effectuée à 3 AN / ml de concentrations optimales d'Artémia.

Mots clés: les facteurs biotiques, le Farfantepenaeus notialis, la croissance, la survie, le Cameroun

*Corresponding author email: Guegang Tekou, email : tekouguegang@yahoo.com

Introduction

World production of shrimp today is growing rapidly and contributing to the expansion of aquaculture. Aquaculture farms provide 20 to 25% of the world's marine shrimp production (Avalle *et al.,* 2003 ; CSAO, 2006). Asia is the largest supplier of shrimp in the world with 88% of production, 41% from China alone (CSAO, 2006). The rest (12%) is largely provides by the Latin America (FAO, 2010). Despite the many possibilities that Africa abounds, shrimp farming is recent (ACP Fish II, 2011). In 2004, total African shrimp production amounted to 8,000 tons, or 0.3 % of total world aquaculture production (ACP Fish II, 2011). In most cases, African farms produce the shrimp Penaeus monodon, highly appreciated in the world market for its growth performances (CSAO, 2006). The major shrimp farming sites in operation in Africa are located mainly in Madagascar, Mozambique, Seychelles and Gambia (CSAO, 2006).

In Cameroon the data available on the shrimp fishery were merely within the marine capture (MINEPIA/DPA, 2009). Despite the production of freshwater shrimp; shrimp production decreased from 2000 tons in 1972 to around 250-450 tons in 2006 (MINEPIA/DPA, 2009; ACP Fish II, 2011). This drastic decline in shrimp production is the result of overexploitation, climate change, pollution and destruction of mangroves for shrimp spawning grounds; associated with rapid population increase (MINEPIA/DPA, 2009). Farfantepenaeus notialis, marine shrimp native to Cameroon, have been dramatically overexploited by local and foreign artisanal fishermen as well as industrial fishermen. The official captures decreased from 35,000 mt in 1999 to 11,000 mt in 2010 (Gaudin *et al.,* 2013). Consequently, the species has a high commercial value on the national market, which would help the successful development of its aquaculture (Gaudin *et al.,* 2013). Moreover, this species has a beautiful appearance that makes it attractive to consumers (Gaudin *et al.,* 2013). In this context, the need for domestication and biodiversity conservation of endogenous shrimp was imposed (Tomedi *et al.,* 2015). Thus in 2007, a collaboration agreement signed between AquaSol-SA and IRAD has allowed the establishment of a shrimp hatchery in the IRAD Station of Kribi (Southern Cameroon) (Njifonjou and Mialhe, 2009; Penkem, 2011; Kenfack, 2012, Gaudin *et al.,* 2013).

Furthermore, domestication and breeding of a species depends on the knowledge of the factors promoting its growth and survival (Fontaine *et al.,* 2009). The breeding success of all living species implies a good control of its environment and biotic factors. According to Cacot and Lazard (2009), the feeding of a species affects in its aquaculture potential and represents 35-40 % of the production cost. Knowing that the basis of the shrimp culture relies on the availability of larvae / post larvae (Alvarez *et al.,* 2004; Kenfack, 2012), control of food at different larval stages could contribute to sustainable post-larvae production and lower cost. In Cameroon, some studies have already been conducted in captivity on the effects of exogenous factors on shrimp reproduction and growth to the Mysis, post-larvae and juvenile stages (Penkem, 2011; Kenfack, 2012; Tekou; 2013; Nwamo *et al.,* 2014; Tekou *et al.,* 2014 and Tomedi *et al.,* 2015). To our knowledge no study has been done on the effects of biotic factors on the breeding of post-larval penaeid. This work is therefore devoted to determine the Artemia (Artemia salina) optimal concentration as feeding of marine shrimp Farfantepenaeus notialis (Pérez Farfante, 1967) of Zoe 3 to Post larve 1 stages in captivity.

Material and methods

Study area

The study was carried out in the AQUASOL SA structure, IRAD Kribi, Department of Ocean in Southern Cameroon region (latitude 2°56'N et longitude 9°54'E), between July and August 2013. The climate is classic guinean type with maritime predominance. It offers two main climatic shades: maritime nuance and shade within Guinea, respectively introduced by the proximity of the sea and continentality (MEAO,

2003). In fact, the oceanian climate is warm and rainy. There are four seasons reducing in the coastal area into two main seasons: a long rainy season (March to October) and a long dry season (November to February); there are no completely dry months. Average rainfall and temperature are respectively 2970 mm and 26 °C. This Department is characterized by a particularly dense hydrographical network, with many rivers (Kienké, Lokoundjé, Lobe, Nyong and Ntem), most of which are rooted in the South Cameroon Plateau and all flow into the Atlantic Ocean (MEAO, 2003). The relief of the continental shelf of Kribi is hilly because of rocky banks and mounds of sand; soils are hydromorphic kind of ferralitic in floodplains (Letouzey, 1969). The hatchery is located in the border of the sea and far enough away from rivers joining it to prevent the influx of fresh water (greater than 25 ppt salinity) and water too loaded with suspended solids.

Animal material and food used

Ethics

Just as shrimp, brine shrimp are not classified among the protected species nor in Cameroonian law either in international law. Furthermore, throughout the study, these two species have been subject to no handling of torture. This study was designed quite simply to improve their production in captivity, in order to no longer depend on the natural environment.

3600 larvae from a pregnant female (body weight 70.6 g and total length 20 cm), collected from a fisherman were fed with live food composed of 5 g of Artemia salina cysts (imported from salt lakes of Salina in the state of Utah in the USA) and microalgae of the species Thalassiosira pseudonana, cultivated in AquaSol SA- IRAD station. These living foods were supplemented by dry food to 53% crude protein, of Creve Tec brand and imported from SORGAL society in Portugal.

Experimental design

The experimental design (Table 1) consisted of 6 treatments with 3 replicates each. Treatments were randomly distributed into 18 cylinder-conical plastic bottles (recovered materials) from the brewing company SuperMont. These bottles 10 L of capacity were cut at their base and filled with 4 l of sea water each. The device was also equipped with a closed-circuit water heater at 34°C.

Assay conduct

The seawater used in this assay was pumped prior, decanted, filtered to 5 µm. and then introduced into a 100 liter tank. This water was treated with EDTA (10 mg / l), sterilized with chlorine (1 ml / l) and maintained at strong aeration for at least 5 hours before use.

The pregnant female was introduced into a spawning tank (30 ° C and 26 ppt salinity) on arrival at 9 am 30. Spawning took place at 2 am next day and eggs were counted. They were then transferred to an incubation tray 2 am 50 (30 ° C and 26 ppt salinity). Hatching had occurred at 10 am and larvae were also counted. 48 hours post hatching; larvae (Nauplii 5) were transferred to the basins of larval rearing. They were fed on microalgae and dry food until Zoe 3 stage (after 6 days). Then 200 larvae of Zoe 3 stage were introduced into each bottle by density of 50 Zoe / l. The total length and number of metamorphosis of a sample of 10 randomly selected larvae were determined in advance.

Zoe 3 larvae were fed to Artemia 2 times a day within an interval of 12 hours (08 am and 20 pm), to dry food 3 times / day in a 8 hours interval of time (10 am, 18 pm and 2 am) and to microalgae 1 time / day (morning at 8 am 00). Artemia cysts were previously hydrated, decapsulated, incubated, preserved (7 days minimum to 4 ° C) and calculated volumetric before distribution using a micro pipette (10-100 µl) MICROLIT brand. The dry foods were weighed using an electronic balance 10-3 g accuracy brand Sartorius Competence and distributed using a Pasteur pipette (5 ml) and a graduated plastic test tube (50 ml). Living

microalgae were distributed to the graduated plastic test tube (50 ml).

Each day from 8pm, 05 larvae were taken at random from each replicate, then introduced into 50 ml plastic test tubes and 03 drops of Betadine were added thereto. Their total lengths and their larval stages were then identified. Salinity, temperature and pH of the water were sampled 02 times per day to 5am 00 and 8pm 00, during the study using respectively a brand salinometer HANNA, a thermometer and a JBL pH-paper. The average values of temperature, salinity and pH were; 30.50 ± 0.04 ‰, 31.00 ± 0.53 ° C and 7.80 ± 0.01 ppt respectively. The different replicates were siphoned daily from 5 pm 30 and the water was then renewed at 50%. Siphoned water was evacuated through a screen of 180 µm mesh, which allowed to retain larvae in experimentation.

Data collected

The total length was determined by optical microscope brand ZEIZZ (magnification × 5 cm and precision 10-1 cm). 05 larvae per replicate were randomly selected and measured each of the front edge of the carapace at the end of the telson (Gaxiola *et al.,* 2010).

The larval stage of each larva was determined using the morphological description key of Dobkin (1961). The number of larval Zoe 3 and post-larvae 1 stages was determined by a visual count. Quantity distributed of Artemia by treatment was assessed using a micro pipette (10-100 µll) of MICROLIT brand. The cost of feed per kilogram was also determined (Price in Kilogram converted to milligram).

Studied parameters

The following parameters were studied:

- Survival rate (SR) was calculated using the formula : SR (%) = (Nf/Ni) X 100, with Ni and Nf : number of shrimp at the beginning and at the end of experiment respectively)
- Metamorphosis rates also called development index (ID), was calculated using Villegas & Kanazawa (1980) formula: ID=(∑□A)/N With A = absolute value X no. Where no = number of larvae in each considered stage; N = total number of larvae in each sample. Absolute values assigned to each sub stage are: Zoe 3 = 3; Mysis 1 = 4; Mysis 2 = 5, Mysis 3 = 6; Post-larve1 = 7.
- Length gain (LG) between the beginning and end of the study was calculated according to the formula used by Gaxiola et al. (2010): LG (mm) = TLf -TLi , where imTL (mm) = initial mean total length and fmTL (mm) = final mean total length
- Mean daily length gain (mDLG) was calculated using the formula: mDLG = (fmTL – imTL) / t, With t : time in day.
- Specific growth rates (SGR) was calculated according to (Ricker, 1975) formula : (SGR (%) = 100 X [Ln fmTL – Ln imTL)] / t, where Ln : logarithm Neperian) .
- Food cost : The quantity and cost of imported dry food as well as those living microalgae are fixed for all treatments, only the concentration of Artemia varies. The cost of dry food (FC) is calculated using the formula: FC (f CFA) = Quantity of dry food distributed × Price of food by Kilogram

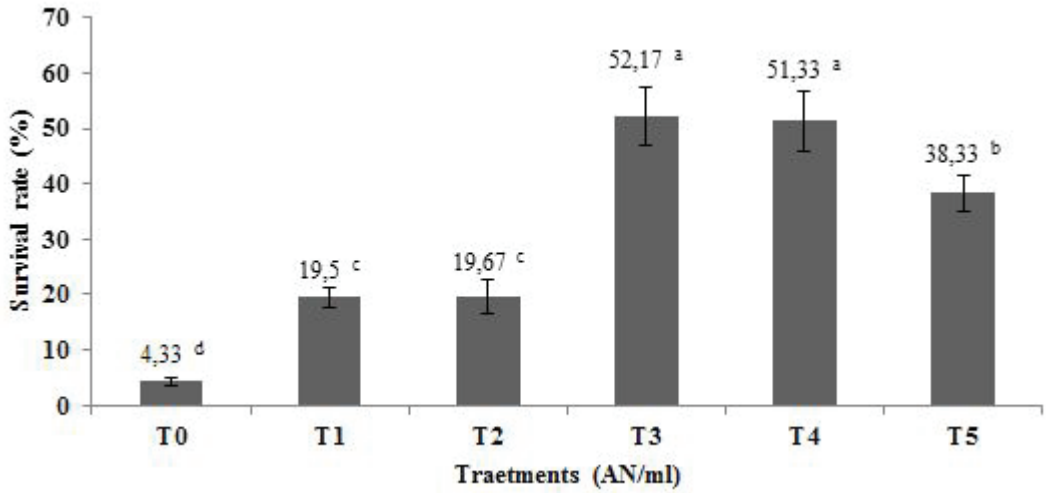
Statistical analysis

Data were subjected to analysis of variance (ANOVA I), and when the differences were significant, means were separated using the Duncan test at 5% probability level. SPSS 20.0 software was used for this analysis.

Results

Survival rates of F. notialis larvae from Zoe 3 to post-larvae 1 stages depending on Artemia concentrations in captivity

Figure 1 shows the survival rate of F. notialis larvae at different concentrations of Artemia. It appears that survival rates between treatments were low (< 55%) and significantly different ($p < 0.05$). However, the T3 et T4 concentrations showed similar results ($P = 0.05$) and they were significantly higher ($P <$



(a, b, c) : each column with same superscript were not significantly different (P □ 0.05) :
T0 : 0 NA/ml,T1 : 1 NA/ml,T2 : 2 NA/ml,T3 : 3 NA/ml,T4 : 4 NA/ml,T5 : 5 NA/ml, NA :Artemia Nauplii

Figure 1: Average survival rates of F. notialis in captivity depending on Artemia concentrations

0.05) than others.

2- Growth performances of F. notialis from Zoe 3 to Post-larves stages 1 depending on Artémia concentrations in captivity

From Table 1 shows growth performances of F. notialis larvae depending on concentrations of Artemia, it appears that shrimp fed at 3 AN / ml recorded higher growth performances. However, these performances were comparable (P = 0.05) to those shrimp fed on 4 AN / ml concentrations of Artemia. Furthermore; these performances are significantly higher than those of other batches. The witness treatment T0 recorded the weakest growth performances (P < 0.05).

Effect of Artemia concentrations on metamorphosis rates of F. notialis from Zoe 3 to Post-larves 1 stages

Table 3 shows that regardless the treatments, the highest transformation rates (p <0, 05) were observed on days 3 and 4 which corresponding to the Mysis 3 and Post-larva 1 stages. Depending on the treatment and independently of periods, the highest metamorphosis rate (p <0, 05) were recorded from T3. The lowest metamorphosis rate from Mysis 3 to Post-larva 1 (day 4) corresponds to 0 AN / ml.

Food cost (FC) depending on concentrations of

Table 1: Different treatment depending on the concentrations of Artemia in AN / ml distributed in replicates per daily ration per meal.

Traitements	Concentrations of Artémia (AN/ml)	
	per daily	Per meal
T0	0	0,0
T1	1	0,5
T2	2	1,0
T3	3	1,5
T4	4	2,0
T5	5	2,5

AN :Artemia Nauplii

Table 2 : Growth performances of F. notialis in captivity depending on Artemia concentrations

Treatments	Growth performances				
	iTL (mm)	fTL (mm)	TLG (mm)	mDGL(mm)	SGR (%)
T0	2,56	2,99 ± 0,09^{c}	0,43 ± 0,10^{c}	0,09 ± 0,02^{c}	1,35 ± 0,26^{c}
T1	2,56	3,18 ± 0,08^{bc}	0,62 ± 0,09^{bc}	0,12 ± 0,02^{bc}	1,89 ± 0,21^{bc}
T2	2,56	3,16 ± 0,19^{bc}	0,60 ± 0,23^{bc}	0,12 ± 0,04^{bc}	1,81 ± 0,53^{bc}
T3	2,56	3,63 ± 0,18^{a}	1,07 ± 0,22^{a}	0,21 ± 0,04^{a}	3,03 ± 0,43^{a}
T4	2,56	3,57 ± 0,05^{a}	1,01 ± 0,06^{a}	0,20 ± 0,01^{a}	2,88 ± 0,11^{a}
T5	2,56	3,39 ± 0,04^{ab}	0,83 ± 0,05^{ab}	0,17 ± 0,01^{ab}	2,44 ± 0,11^{ab}
Means	2,56	3,32 ± 0,26	0,76 ± 0,26	0,15 ± 0,05	2,23 ± 0,68

Mean ± standard deviation is based on three replicates, (a, b, c) : each column with same superscript were not significantly different (P < 0.05) , T0 : 0 AN/ml, T1 : 1 AN/ml, T2 : 2 AN/ml, T3 : 3 AN/ml, T4 : 4 AN/ml, T5 : 5 AN/ml, AN : Artemia Nauplii , iTL : initial total length, fTL : final total length, LGT : total gain length, mDLG : mean daily gain and SGR : spécific growth rate.

Table 3 : Daily evolution of metamorphosis rate of F. notialis depending on different artemia concentrations in captivity

	Treatments					
Days	T0	T1	T2	T3	T4	T5
J0	3,00 ± 0,00^{a}	3,00 ± 0,00^{a}	3,00 ± 0,00^{a}	3,00 ± 0,00^{a}	3,00 ± 0,00^{a}	3,00 ± 0,00^{a}
J1	1,87 ± 0,92^{b}	1,60 ± 0,00^{b}	1,87 ± 0,46^{b}	3,20 ± 0,00^{a}	1,87 ± 0,46^{b}	2,13 ± 0,92^{b}
J2	2,40 ± 0,00^{b}	1,67 ± 0,58^{b}	1,67 ± 1,15^{b}	3,67 ± 0,58^{a}	2,33 ± 0,58^{b}	2,33 ± 0,57^{b}
J3	2,4 ± 0,00^{b}	1,6 ± 0,70^{b}	2,00 ± 0,70^{b}	4,40 ± 0,70^{a}	2,80 ± 0,07^{b}	2,80 ± 0,70^{b}
J4	0,93 ± 0,80^{b}	1,40 ± 1,40^{b}	1,87 ± 0,81^{ab}	3,73 ± 0,81^{a}	3,73 ± 1,62^{a}	3,73 ± 0,81^{a}

Mean ± standard deviation is based on three replicates, (a, b, c) : each column with same superscript were not significantly different (P □ 0.05) , T0 : 0 AN/ml, T1 : 1 AN/ml, T2 : 2 AN/ml, T3 : 3 AN/ml, T4 : 4 AN/ml, T5 : 5 AN/ml, AN : Artemia Nauplii ; J0 : day 0 (Zoea 3), J1 : day 1 (Mysis 1), J2 : day 2 (Mysis 2), J3 : day 3 (Mysis 3) and J4 : day 4 (Post-larvae 1).

Table 4 : Food cost depending to Artemia concentrations and quantities of food distributed for rearing F. notialis from Zoea 3 to Post-larvae 1 stages in captivity

	Treatments					
Food cost and quantities	T0	T1	T2	T3	T4	T5
Food cost (FCFA)	22,57	49,41	76,25	103,09	129,92	156,77
Quantity (mg)	668,30	896,40	1124,60	1352,70	1580,80	1809,00
Unit cost (FCFA/mg)	0,03^{c}	0,06^{b}	0,07^{b}	0,08^{ab}	0,08^{ab}	0,09^{a}
Unit cost (FCFA/g)	30,00^{c}	60,00^{b}	70,00^{b}	80,00^{ab}	80,00^{ab}	90,00^{a}

($^{a, b, c}$) : each line with same superscript were not significantly different (P □ 0.05), T0 : 0 AN/ml, T1 : 1 AN/ml, T2 : 2 AN/ml, T3 : 3 AN/ml, T4 : 4 AN/ml, T5 : 5 AN/ml, AN : Artemia Nauplii

Artemia

The cost of feed (dry feed and Artemia) depending on the concentration of Artemia as presented in Table 4 shows that, the unit cost of feed increases with Artemia concentrations (p < 0, 05). However, the T3 and T4 treatments recorded comparable unit costs (P = 0.05).

Discussion

The lowest survival rate recorded by the witness treatment T0 showing the influence of Artemia on larval survival. Registered survival rates were low (< 55%) and the highest was 52.17%, corresponding to 3 AN / ml. These results do not corroborate with those of Gaxiola et al. (2010) who reported from F. brasilensis the highest survival rate of 98.3 ± 2.9% to 4AN/ml. This superiority could be due to differences in experimental conditions (Alvarez *et al.,* 2004). Indeed, these authors worked at a water temperature of 28 ° C and a salinity of 35 ppt, while in this study, these parameters were 30.5 ° C and 31 ppt respectively. However, our study only respected the optimal conditions of salinity (between 20 and 38 ‰) as indicated in technical sheet (2014).

Growth performances of the control treatment (0 AN / ml) are lowest. This shows the positive impact of live food on linear growth in larval rearing. Indeed, Barros and Valenti (2003) show that in hatchery, due to the higher demand of larvae for their growth, it is necessary to supplement commercial food with Artemia. Treatment with the highest growth performances corresponds to 3 AN / ml. These results are different from those of Gaxiola et al. (2010) who found no significant difference between treatments. Furthermore, these authors obtained 4 AN / ml concentration as having highest growth performances. These differences could be explained by factors relating to the species used. In fact, Gaxiola et al. (2010) experimented on species Farfantepenaeus brasiliensis and Artemia franciscana contrary to this essay which was carried out on Farfantepenaeus notialis and Artemia salina.

The metamorphosis rate from Mysis 3 to Post-larva 1 (day 4) was the lowest (0.93 ± 0.81) to 0AN / ml. This result could be explained through the work of Barros et Valenti (2003) which showed that during the preparation of larval to their metamorphosis for Post-larva 1, energy demand is high. Hence the need for the use of Artemia for energy intake. In 4 days, the concentration of 3 AN/ml gave the highest metamorphosis rate from Mysis 1 to Post-larva 1 stages (3,20 ± 0,00 ; 3,67 ± 0,58 ; 4,40 ± 0,70 et 3,73 ± 0,81 respectively). These results are different from those of Gaxiola et al. (2010), who found no significant difference between treatments. Furthermore in four days they have registered the highest metamorphosis rate (4 ± 0 ; 5 ± 0 ; 5,7 ± 0,33 et 7 ± 0) with 4 AN/ml. This divergence could be explained by the fact that these authors have combined the Artemia and diatoms (Chaetoceros gracilis : 80 × 103 cells/ml) and flagellates (Tetraselmis chuii: 2 × 103 cells/ml). While in this study, they are combined with the commercial feed (formulated to 53% protein) and diatoms (Thalassiossira pseudonana).

Treatments T3, T4 and T5 cost respectively 3 and 2.7 times more expensive than the treatment T0. These results are similar to those of Banag (2012) which showed that the living food cost relatively more expensive than artificial foods. Robinson et al. (2005) in the same direction show that the use of artificial feed for the production of shrimp larvae would represent a significant reduction in production costs. This insofar as the use of artificial food reduces or eliminates the costs associated with microalgae or Artemia cultures.

Conclusion

On completion of this study, it appears that supplementation of Artemia nauplii in shrimp feeding from Zoe 3 to Post larvae stages has a positive influence on their survival, growth performance and metamorphosis rates. It also reveals that the concentration of 3 AN / ml of Artemia salina is optimum for larval culture of Farfantepenaeus notialis, since it gives the best performances for survival, growth, metamorphosis and minimizes the cost of production, compared to other concentrations.

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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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- *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.
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