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BLOOD BIOCHEMICAL CONSTITUENTS OF GESTATING RABBIT DOES AS AFFECTED BY VITAMIN *E INCLUSION* AND PERIODS OF FEED RESTRICTION DURING PREGNANCY

Adeyemo A A[1]* Sogunle O M[1], Adeyemi O A[1] and Bamgbose A M[2]
[1]Department of Animal Production and Health,
[2]Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta

Abstract

Blood plays a vital role in the physiological, nutrition and pathological status of an organism (Aderemi, 2004; Doyle, 2006). The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals.. Thus, this study aimed to evaluate the effect of feed restriction during pregnancy with or without vitamin E on the blood biochemical constituents of gestating rabbit does. A total of Seventy five (75) rabbits consisting of sixty (60) 20 weeks old does of mixed breeds (Chinchilla, Dutch and New Zealand) with initial live weights of 1.7-2.0 kg were randomly assigned into 12 treatment groups of 5 replicates each during pregnancy. The rabbit does were exposed to two levels of quantitative feed restriction (0% and 15%) at three different periods of gestation (15-19 days, 20-24 days and 25-29 days) with or without vitamin *E inclusion* (0 and 300mg/kg). Data obtained on their blood biochemical indices were subjected to Analysis of Variance in a completely randomized design. Significantly ($P<0.05$) higher total protein and albumin after kindling was obtained for gestating rabbit does on 15% and 0% restriction without vitamin *E inclusion* at 25-29 days and 15-19 days of gestation respectively. Although significant, it is within the range of values reported by some authors as baseline data for blood values in growing rabbits. Hence, it can be concluded that feed restriction and periods of feed restriction with or without vitamin *E inclusion* does not have any negative or detrimental effects on blood biochemical constituents of gestating rabbit does.

Keywords: Feed restriction, blood biochemical parameters, Vitamin E, Pregnant Rabbits.

L'EFFET PRODUIT PAR L'INCLUSION DE LA VITAMINE E ET LES PÉRIODES DE RESTRICTION ALIMENTAIRE DURANT LA GESTATION SUR LES CONSTITUANTS BIOCHIMIQUES SANGUINS DE LAPINES GRAVIDES

Résumé

Le sang joue un rôle vital dans l'état physiologique, nutritionnel et pathologique d'un organisme (Aderemi, 2004; Doyle, 2006). L'examen du sang permet d'étudier la présence de plusieurs métabolites et d'autres constituants dans le corps des animaux. Ainsi, cette étude avait pour objectif d'évaluer l'effet de la restriction alimentaire pendant la gestation avec ou sans inclusion de vitamine E sur les constituants biochimiques sanguins de lapines gravides. Au total, soixante-quinze (75) lapines dont soixante (60) âgées de 20 semaines et de races mixtes (Chinchilla, Néerlandaise et Nouvelle-Zélande) ayant un poids variant entre 1,7 et 2,0 kg ont été réparties de manière aléatoire en 12 groupes de traitement de 5 répétitions chacun pendant la gestation. Les lapines ont été exposées à deux niveaux de restriction alimentaire quantitative (0% et 15%) à trois périodes de gestation différentes (15-19 jours, 20-24 jours et 25-29 jours) avec ou sans inclusion de vitamine E (0 et 300 mg / kg). Les données obtenues sur leurs indices biochimiques sanguins ont été soumises à une analyse de variance dans un schéma complètement randomisé. Des protéines totales et de l'albumine significativement ($P <0,05$) plus élevées ont été obtenues pour les lapines gestantes soumises aux restrictions alimentaires à 15% et 0% sans inclusion de vitamine E, respectivement à 25-29 jours et 15-19 jours de gestation. Bien que significatives, ces valeurs se situent dans la fourchette des valeurs rapportées par certains auteurs comme données de référence pour les valeurs sanguines des lapins

*Corresponding author: arkeens25@gmail.com.

en croissance. Par conséquent, on peut conclure que la restriction alimentaire et les périodes de restriction alimentaire avec ou sans inclusion de vitamine E n'ont pas d'effets négatifs ou néfastes sur les constituants biochimiques sanguins des lapines en gestation.

Mots-clés : restriction alimentaire, paramètres biochimiques sanguins, vitamine E, lapines gravides

Introduction

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood, the red blood cells (*erythrocytes*), white cells (*leucocytes*), and the platelets (*thrombocytes*) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). According to Olafedehan *et al*. (2010), examining blood for its constituents can provide important information for the diagnosis and prognosis of diseases in animals. Blood constituents change in relation to the physiological conditions of health (Togun *et al*., 2007). Haematological studies are important because blood is the major transport system of the body, and evaluations of the haematological profile usually furnishes vital information on the body's response to injury of all forms, including toxic injury (Schalm *et al*., 1975; Coles, 1986; Ihedioha *et al*., 2004). A readily available and fast means of assessing the clinical and nutritional health status of animals on feeding trials may be the use of blood analysis, because the ingestion of dietary components has measurable effects on blood composition (Church *et al*., 1984; Maxwell *et al*., 1990) and may be considered as an appropriate measure of long term nutritional status (Olabanji *et al*., 2007). Vitamin E has been confirmed to possess positive biological actions on growth, regulation of cellular signalling and gene activity, immune system, tissue integrity, and antioxidant capacity (Selim *et al*., 2008, Ebeid *et al*., 2013). Vitamin E is a lipophilic compound that is located in the cell membrane and is particularly efficient at quenching free radicals originating from the mitochondrial inner membrane and other biomembranes (Parker, 1991).

Experimental Site

The experiment was carried out at the Rabbitary Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State. The site is located in the rain forest vegetation zone of South-Western Nigeria on latitude 7°13' 49.46' N, longitude 3°26 11.98' E at an altitude of 76m above sea level. The climate is humid with a mean annual rainfall of 1037 mm and mean temperature and humidity of 34.7°C and 83%, respectively (Google Earth, 2015).

Experimental Animals and Management

Seventy five (75) rabbits consisting of sixty (60) 20 weeks old does of mixed breeds (Chinchilla, Dutch and New Zealand) with initial live weights of 1.7-2.0 kg and fifteen (15) mature bucks with live weights of 2.0-2.5 kg were used for the study. The hutches were washed and disinfected prior to the commencement of the experiment. The does were divided into two groups of thirty (30) rabbits each after balancing for weight and housed individually in hutches with dimensions of 0.8×0.5×0.6 m. The rabbit bucks were similarly housed individually.

Experimental Design

The treatments consisted of two (2) levels of feed restriction (0 and 15%) at three (3) different periods (15-19, 20-24, 25-29 days) during pregnancy with or without vitamin *E inclusion* (0 and 300 mg/kg). The does were divided into 12 groups of 5 replicates of 1 rabbit each.

The treatments consisted of three factors: levels of feed restriction, vitamin E and periods of feed restriction. 0% Restriction (control) was fed at 100 g/rabbit/day. (*Ad libitum* feeding) 15% Restriction was fed at 85 g/rabbit/day.

The feeding recommendation of 100 gof feed for gestating does was based on a previous study at the same location (Adeyemo, 2014). Vitamin *E inclusion* at 300 mg/kg of feed was according to the recommendation of Virág *et al*. (2008) in rabbits.

Rabbits on 0% restriction were offered 100 g of feed with or without vitamin *E inclusion* daily throughout the experimental period of 32 days. Rabbits on 15% restriction were offered 100 grams of feed daily with or without vitamin *E inclusion* before and after the restriction periods, while 85 grams of feed were offered during the periods of feed restriction. The composition of concentrate feed fed to the breeder rabbits is shown on Table 1

Data Collection

The experiment lasted for 32 days, which was the pregnancy period of the rabbit does during which blood samples were collected for blood biochemical constituents.

Blood Analysis

Blood samples were collected at the beginning of the experiment (before mating) and at the end of the experiment (2days after kindling). At the time of blood collection three rabbit does in each level under each period with or without vitamin E were selected for blood collection. Blood samples of 3ml were withdrawn from the ear vein of each doe by means of a sterile hypodermic needle and syringe; 2ml of blood was collected and put into bottles with anti-coagulant for blood serum analysis. The parameters analyzed included serum total protein, albumin and globulin. These parameters were analysed according to the methods of Tietz (1995) and Donmas *et al* (1971).

Statistical Analysis

The experimental layout was in a 2×3×2 factorial arrangement using a completely randomized design using (SAS, 1999). Significantly ($p<0.05$) different means were separated using Duncan's Multiple Range Test of SAS (1999) statistical package.

Ethical Approval: All rules guiding animal welfare and procedures were strictly adhered to following the rules and regulations of the Animal welfare Committee of the College of

Table 1: Composition of concentrate breeder diets

Ingredients (%)	**A**	**B**
Maize	47.50	47.50
Fish meal	2.00	2.00
Soybean meal	3.00	3.00
Wheat offal	23.00	23.00
Groundnut cake	12.00	12.00
Rice husk	7.00	7.00
Bone meal	3.00	3.00
Oyster shell	2.00	2.00
Salt	0.25	0.25
*Vitamin and Mineral premix	0.25	0.25
	100	100
Vitamin E	-Vit.E	+ Vit.E
Determined Analysis		
ME (Kcal/kg)	2578.8	2578.8
Ash (%)	2.74	2.74
Crude fibre %	10.65	10.65
Crude protein	16.20	16.20
Nitrogen free extract	42.50	42.50

** Premix contained: Vit A 8000 iu, Vit D3 2000 iu, Vit E 4000 iu, Vit K 2 mg, Riboflavin 4.20 mg, Vit B12 0.01 mg, Pantothenic acid 5 mg, Nicotinic acid 20 mg, Folic acid 5 mg, Choline 300 g, Mn 56 mg, Fe 20 mg, Cu 10 mg, Zn 50 mg.*

Animal Science and Livestock Production, Federal University of Agriculture Abeokuta.

Results

Table 2 shows the effect of restriction levels, periods of feed restriction and vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does. The results obtained on blood biochemical constituents of gestating rabbit does after kindling shows that there were no significant ($p>0.05$) differences for all the parameters measured for levels and period of feed restriction with or without vitamin *E inclusion* of gestating rabbit does.

The interactive effect between levels and periods of feed restriction on blood biochemical constituents of gestating rabbit does is shown in Table 3 The interactive effect between levels and periods of feed restriction had no significant effect ($p>0.05$) on all parameters measured for blood biochemical constituents of gestating rabbit does except for the total protein of gestating rabbit does before mating.

Table 4 shows the interactive effect between the levels of feed restriction with or without vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does. The interactive effect between levels of feed restriction with or without vitamin *E inclusion* shows that there were no significant ($p>0.05$) differences in all the parameters measured for blood biochemical constituents of gestating rabbit does except for the total protein of gestating rabbits before mating.

The interactive effect between periods of feed restriction with or without vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does is shown in Table 5. The results show that there were no significant ($p>0.05$) differences in all the parameters measured for blood biochemical constituents of gestating rabbit does.

Table 6 shows the interactive effect between levels and periods of feed restriction with or without vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does. Significant ($p<0.05$) differences were obtained on total protein, albumin and globulin levels. The highest mean values (7.00 ± 0.80 %) for total protein was obtained for gestating rabbit does on 15% restriction at 25-29 days of gestation without vitamin *E inclusion* while the least (5.30 ± 1.00 %) was obtained at the same level at 20-24 days of gestation with vitamin *E inclusion*. Albumin was significantly ($p<0.05$) higher (3.90 ± 0.30 %) for rabbit

Table 2: Effect of restriction levels, periods of feed restriction and vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does

Parameters	Levels of feed restriction		Periods of feed restriction			Vitamin inclusion	
	0%	15%	15-19days	20-24days	25-29days	-Vit. E	+Vit. E
T.Protein (g/dl) Before mating	4.80 ± 0.36	5.10 ± 0.56	4.92 ± 0.46	4.85 ± 0.46	5.08 ± 0.55	4.99 ±0.58	4.91± 0.39
T.Protein (g/dl) After kindling	5.99 ± 0.45	6.08 ± 0.77	5.97 ± 0.51	5.75 ± 0.57	6.38 ± 0.64	6.16 ±0.56	5.90 ±0.67
Albumin (g/dl) Before mating	2.62±0.34[b]	2.90± 0.43[a]	2.71± 0.41	2.71±0.39	2.86 ± 0.44	2.82 ±0.50	2.70 ±0.29
Albumin (g/dl) After kindling	3.26 ± 0.38	3.40 0.45	3.53± 0.42	3.16± 0.36	3.30± 0.40	3.45± 0.39	3.21±0.41
Globulin (g/dl) Before mating	2.17 ± 0.06	2.20 ± 0.24	2.21 ± 0.09	2.13 ± 0.20	2.22 ± 0.20	2.16 ±0.18	2.21 ± 0.16
Globulin (g/dl) After kindling	2.72 ± 0.49	2.68 ± 0.41	2.43 ± 0.39	2.58 ± 0.28	3.08 ± 0.39	2.71 ±0.50	2.69 ± 0.40

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

**T.Protein : total protein*

Table 3: Interactive effect between levels and periods of feed restriction on blood biochemical constituents of gestating rabbit does

Levels of feed restriction	**0%**			**15%**		
Periods of feed restriction	**15-19days**	**20-24days**	**25-29days**	**15-19days**	**20-24days**	**25-29days**
Parameters						
T.Protein(g/dl)Before mating	4.90 ± 0.27^{ab}	4.80± 0.41^{b}	4.70± 0.40^{b}	4.95± 0.62^{ab}	4.90 ±0.53^{ab}	5.47 ± 0.39^{a}
T.Protein(g/dl)After kindling	5.77 ± 0.43	5.80 ± 0.19	6.40 ± 0.42	6.17 ± 0.55	5.70 ± 0.83	6.37 ± 0.85
Albumin(g/dl)Before mating	2.72 ± 0.28	2.60 ± 0.35	2.55 ± 0.40	2.70 ± 0.54	2.80 ± 0.42	3.17 ± 0.18
Albumin(g/dl)After kindling	3.45 ± 0.56	3.15 ± 0.11	3.20 ± 0.31	3.62 ± 0.26	3.17 ± 0.53	3.40 ± 0.47
Globulin(g/dl)Before mating	2.17 ± 0.07	2.20 ± 0.07	2.15 ± 0.04	2.25 ± 0.10	2.07 ± 0.27	2.30 ± 0.27
Globulin(g/dl)After kindling	2.32 ± 0.46	2.65 ± 0.28	3.20 ± 0.26	2.55 ± 0.32	2.52 ± 0.30	2.97 ± 0.49

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

**T.Protein : total protein*

Table 4: Interactive effect between levels of feed restriction with or without vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does

Levels of feed restriction	**0%**		**15%**	
Vitamin *E inclusion*	**+Vit.E**	**-Vit. E**	**+Vit. E**	**-Vit.E**
Parameters				
T.Protein(g/dl)Before mating	4.66 ± 0.29^{b}	4.93 ± 0.38^{ab}	5.16 ± 0.32^{a}	5.05 ± 0.75^{ab}
T.Protein(g/dl)After kindling	5.96 ± 0.59	6.01 ± 0.29	5.85 ± 0.77	6.31 ± 0.73
Albumin(g/dl)Before mating	2.51 ± 0.25	2.73 ± 0.39	2.88 ± 0.22	2.91 ± 0.59
Albumin(g/dl)After kindling	3.15 ± 0.27	3.38 ± 0.44	3.28 ± 0.53	3.51 ± 0.35
Globulin(g/dl)Before mating	2.15 ± 0.04	2.20 ± 0.07	2.28 ± 0.20	2.13 ± 0.26
Globulin(g/dl)After kindling	2.81 ± 0.48	2.63 ± 0.51	2.56 ± 0.27	2.80 ± 0.51

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

**T.Protein : total protein*

Table 5: Interactive effect between levels and periods of feed restriction with or without vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does

Levels of feed restriction	**0%**			**15%**		
Periods of feed restriction	**15-19days**	**20-24days**	**25-29days**	**15-19days**	**20-24days**	**25-29days**
Parameters						
T.Protein(g/dl)Before mating	4.87 ± 0.32	4.85 ± 0.43	5.02 ± 0.45	4.97 ± 0.59	4.85 ± 0.53	5.15 ± 0.67
T.Protein(g/dl)After kindling	6.07 ± 0.69	5.47 ± 0.66	6.17 ± 0.51	5.87 ± 0.28	6.02 ± 0.34	6.60 ± 0.74
Albumin(g/dl)Before mating	2.65 ± 0.23	2.62 ± 0.30	2.82 ± 0.35	2.77 ± 0.55	2.80 ± 0.47	2.90 ± 0.55
Albumin(g/dl)After kindling	3.37 ± 0.50	3.07 ± 0.42	3.20 ± 0.31	3.70 ± 0.29	3.25 ± 0.31	3.40 ± 0.47
Globulin(g/dl)Before mating	2.22 ± 0.10	2.22 ± 0.15	2.20 ± 0.23	2.20 ± 0.08	2.05 ± 0.22	2.25 ± 0.19
Globulin(g/dl)After kindling	2.70 ± 0.24	2.40 ± 0.26	2.97 ± 0.47	2.17 ± 0.34	2.77 ± 0.17	3.20 ± 0.28

**T.Protein : total protein*

Table 6: Interactive effect between levels and periods of feed restriction with or without vitamin *E inclusion* on blood biochemical constituents of gestating rabbit does

Levels of feed restriction	**0%**						**15%**					
Periods of feed restriction	**15-19days**	**20-24days**	**25-29days**	**15-19days**	**20-24days**	**25-29days**	**15-19days**	**20-24days**	**25-29days**	**15-19days**	**20-24days**	**25-29days**
Vitamin *E* inclusion	**+Vit. E**	**+Vit. E**	**+Vit. E**	**-Vit. E**	**-Vit. E**	**-Vit. E**	**+Vit. E**	**+Vit. E**	**+Vit. E**	**-Vit. E**	**-Vit. E**	**-Vit. E**
Parameters												
T.Protein(g/dl) Before mating	4.75 ± 0.25^{b}	4.50± 0.10^{b}	4.75 ± 0.45^{b}	5.05±0.25^{ab}	5.10 ±0.40^{ab}	4.65 ± 0.45^{b}	5.00 ± 0.40^{ab}	5.20 ± 0.30^{ab}	5.30 ± 0.30^{ab}	4.90 ± 0.90^{ab}	4.60 ±0.90^{b}	5.65 ±0.45^{a}
T.Protein(g/dl) After kindling	5.65± 0.65^{bc}	5.65±0.05^{bc}	6.60 ±0.30^{ab}	5.90±0.00^{bc}	5.95 ±0.15^{bc}	6.20 ± 0.50^{abc}	6.50 ± 0.50^{ab}	5.30 ± 1.00^{bc}	5.75 ± 0.15^{bc}	5.85 ± 0.45^{bc}	6.10 ±0.50^{abc}	7.00 ±0.80^{a}
Albumin(g/dl) Before mating	2.60 ± 0.20^{ab}	2.35±0.05^{b}	2.60±0.40^{ab}	2.85 ±0.35^{ab}	2.85 ±0.35^{ab}	2.50 ± 0.05^{b}	2.70 ± 0.30^{ab}	2.90 ± 0.10^{ab}	3.05 ± 0.05^{ab}	2.70 ± 0.08^{ab}	2.75 ±0.65^{ab}	3.30 ±0.20^{a}
Albumin(g/dl) After kindling	3.00± 0.30^{dc}	3.25 ±0.00^{abcd}	3.20± 0.40^{bcd}	3.90 ± 0.30^{a}	3.05 ± 0.05^{dc}	3.20 ± 0.30^{bcd}	3.75 ± 0.35^{ab}	2.90 ± 0.65^{d}	3.20 ± 0.30^{bcd}	3.50 ± 0.10^{abcd}	3.45 ±0.35^{abcd}	3.60 ±0.60^{abc}
Globulin(g/dl) Before mating	2.15 ± 0.05	2.15 ± 0.05	2.15 ± 0.05	2.20 ± 0.10	2.25 ± 0.05	2.15 ± 0.05	2.30 ± 0.10	2.30 ± 0.20	2.25 ± 0.35	2.20 ± 0.10	1.85 ± 0.05	2.35 ± 0.25
Globulin(g/dl) After kindling	2.65± 0.35^{bcd}	2.40±0.10^{cd}	3.40 ± 0.10^{a}	2.00 ± 0.30^{e}	2.90 ± 0.10^{bc}	3.00 ± 0.20^{ab}	2.75 ± 0.15^{bcd}	2.40 ± 0.40^{ed}	2.55 ± 0.15^{cd}	2.35 ± 0.35^{ed}	2.65 ± 0.15^{bcd}	3.40 ± 0.20^{a}

$^{a, b, c, d}$ *:Means in the same row with different superscripts differ significantly ($p<0.05$)*

**T.Protein : total protein*

does on 0% restriction between 15-19 days of gestation without vitamin *E inclusion* compared to 15% restriction at 20-24 days of gestation with vitamin *E inclusion* that recorded the lowest mean value (2.90 ±0.65 %). Globulin levels were significantly ($p<0.05$) higher (3.40 ± 0.10 % and 3.40 ± 0.20 %) in rabbits on 0% and 15% restriction between 25-29 days of gestation with and without vitamin *E inclusion* respectively.

Discussion

The effects of restriction levels, periods of feed restriction and vitamin *E inclusion* on the blood biochemical constituents of gestating rabbit does showed that there were no significant differences in all the parameters measured. The results of the total protein, albumin and globulin levels across the dietary treatments though not significant, were slightly higher than values reported by Ebeid *et al*. (2013) in growing rabbits. The slightly higher mean values obtained for this study may be attributed to the physiological state of the rabbits (gestating does). Also, the results obtained on globulin with or without vitamin *E inclusion* though not significant, are contrary to the work of Ebeid *et al*. (2013) who reported significant differences in the globulin levels of growing rabbits with vitamin *E inclusion*.

The interactive effects between levels and periods of feed restriction with or without vitamin *E inclusion* on the blood biochemical constituents of gestating rabbit does showed significant differences in the total protein, albumin and globulin levels. The results obtained on these parameters could not be attributed to the treatment effect. The mean values obtained in this study though significant are higher than those reported by Ebeid *et al*. (2013) in growing rabbits fed an *ad libitum* basal diet with or without vitamin E. The slightly higher mean values obtained in this study could be attributed to the stage of production of the rabbits compared to growing rabbits.

Conclusion

It can be concluded that feed restriction and periods of feed restriction with or without vitamin *E inclusion* do not have any detrimental effects on the blood biochemical constituents of gestating rabbit does as the results obtained in this study were within the ranges of base line blood values reported by authors who have worked on blood biochemical constituents in growing rabbits.

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FIRST REPORT OF AVIAN INFECTIOUS LARYNGOTRACHEITIS OUTBREAK IN SMALL SCALE CHICKEN FLOCKS AROUND HAWASSA CITY, ETHIOPIA

Berhanu Mekibib[1]*, Sisay Dejene[2] and Rahmeto Abebe[1]
[1]Faculty of Veterinary Medicine, Hawassa University, P.O. Box 05
[2]Field Veterinarian, Dore Bafano, Sidama Zone, SNNPRS

Abstract

The growing small scale poultry production in most developing countries is challenged by several factors including diseases of varied etiologies. This study aimed to describe the first outbreak of infectious *Laryngotracheitis* (AILT) in exotic layer chickens managed under semi-intensive production systems in Ethiopia. Outbreak investigations were made between June and July, 2018 following a report of a severe disease outbreak in three small scale poultry farms located at Dore Bafano kebele, Sidama zone. A team of veterinarians then traveled to the area to undertake physical examinations, collect the history and circumstantial evidences and representative chickens for further examinations. To characterize the lesions, systematic postmortem examinations and histopathology were carried out on eight critically sick and four recently dead chickens. The dominant clinical signs were extension of the neck, long-drawn-out gasps, gurgling, rattling, coughing and death after 5 to 8 days of illness. The morbidity and mortality rates were about 98% and 80%, respectively. The mortality rate reached a peak within two days of onset and then gradually declined but continued for 2 weeks. The gross lesions were restricted to the upper respiratory tracts and ranged from hemorrhagic tracheitis, mucoid rhinitis, and blood-stained mucus along the length of the *trachea* of critically sick chickens to diphtheritic or caseous necrotic plaques and plugs in the *trachea*, *larynx* and mouth of recently dead chickens. The hallmark microscopic lesions were erosion and ulceration of the *tracheal mucosa*, *lymphohistocytic* and heterophilic inflammatory infiltrates of the sub*mucosa* and eosinophilic intranuclear inclusion bodies in most of the *tracheal* epithelial cells forming syncytia. Based on the collected information at different levels and while awaiting the results of virus isolation and molecular investigation, the current investigation discovered the outbreak of Avian Infectious *Laryngotracheitis* for the first time in Ethiopia. The virus/disease may have entered the country through contaminated crates along with the importation of day old chicks and remained undetected or misinterpreted because of the poor veterinary services and reporting systems.

Key words: Infectious *Laryngotracheitis*, first report, histopathology, improved chickens, Ethiopia

PREMIER RAPPORT DU FOYER DE LARYNGOTRACHÉITE INFECTIEUSE AVIAIRE DANS LES ÉLEVAGES ARTISANAUX DE POULETS AUTOUR DE LA CITÉ DE HAWASSA EN ÉTHIOPIE

Résumé

La production croissante des élevages artisanaux de volailles dans la plupart des pays en développement est confrontée à plusieurs défis, notamment les maladies d'étiologies variées. Ce rapport avait pour objectif de décrire le premier foyer de laryngotrachéite infectieuse (AILT) chez des pondeuses exotiques élevées en systèmes de production semi-intensifs en Éthiopie. Les investigations sur le foyer ont été menées entre juin et juillet 2018 à la suite d'un rapport faisant état d'une grave épidémie de la maladie dans trois petites exploitations avicoles situées dans la kebele de Dore Bafano, dans la zone de Sidama. Une équipe de vétérinaires s'est ensuite rendue dans la région pour procéder à des examens physiques, recueillir les données sur les antécédents et les éléments circonstanciels et prélever un échantillon de poulets représentatifs pour des examens plus approfondis. Pour réaliser une caractérisation des lésions, des examens post mortem systématiques et histopathologiques ont été effectués sur huit poulets gravement malades et quatre poulets récemment morts. Les signes cliniques dominants étaient l'extension du cou,

*Corresponding author email: berhanumm2002@gmail.com

des halètements prolongés, des gargouillements, des cliquetis, une toux et la mort après 5 à 8 jours de maladie. Les taux de morbidité et de mortalité étaient respectivement d'environ 98% et 80%. Le taux de mortalité a atteint un pic dans les deux jours suivant l'apparition de la maladie, puis a progressivement diminué, mais a continué pendant 2 semaines. Les lésions macroscopiques caractéristiques étaient limitées aux voies respiratoires supérieures et variaient entre la trachéite hémorragique, la rhinite mucoïde et du mucus taché de sang le long de la trachée des poulets gravement malades et les plaques et bouchons nécrotiques diphtériques ou caséeux dans la trachée, le larynx et la bouche des poulets récemment morts. Les lésions microscopiques caractéristiques étaient l'érosion et l'ulcération de la muqueuse trachéale, les infiltrats inflammatoires lymphohistocytaires et hétérophiles des sous-muqueuses et les corps d'inclusion intranucléaires éosinophiles dans la plupart des cellules épithéliales trachéales formant des syncytiums. Sur la base des informations recueillies à différents niveaux et en attendant les résultats de l'isolement du virus et de l'enquête moléculaire, l'investigation en cours a découvert pour la première fois en Éthiopie un foyer de laryngotrachéite infectieuse aviaire. Le virus / la maladie peut avoir été introduite dans le pays via des caisses contaminées en même temps que l'importation de poussins d'un jour, et est restée non détectée ou mal interprétée en raison de la médiocrité des services vétérinaires et des systèmes de notification.

Mots-clés : Laryngotrachéite infectieuse, premier rapport, histopathologie, poulets améliorés, Éthiopie

Introduction

Poultry production in Ethiopia is characterized predominantly by the traditional scavenging system, where 97% is indigenous breed and contributes more than 98% of the total meat and egg production (Udo *et al*., 2006). However, with the increasing demand of affordable protein source, job for new young graduates and the alarmingly increasing human population at large, the number of small scale poultry farms in Ethiopia is surprisingly increasing in recent years (Habte *et al*., 2017).

These farms are stocked with flocks of high producing exotic breeds that were raised and/or distributed at young age by government owned poultry multiplication centers, nongovernmental organizations and private individuals (Pagani and Wossene, 2008; Mengesha, 2012). Despite this promising advance, the poultry industry, particularly the small scale exotic poultry production system, is confronted with a variety of problems including availability of affordable feed, adequate space, limited access to veterinary inputs, biosecurity issues and diseases of infectious and noninfectious origin (Sambo *et al*., 2015).

Infectious diseases, particularly of the viral origin, are widely accepted by producers and researchers as one of the major bottleneck to the developing poultry production. Newcastle disease (Tadesse *et al*., 2005; Mazengia *et al*., 2010; Chaka *et al*., 2012), Infectious bursal disease (Zeleke *et al*., 2005, Mazengia, 2009), and Marek's disease (Duguma *et al*., 2005) are among the diseases widely present and reported in the country. Moreover, a recent molecular and serological screening made by Hutton *et al*. (2017) in one of the governmental poultry production and distribution center located in Debre Zeit (central Ethiopia) indicated the presence of Infectious bronchitis among other bacterial and viral respiratory pathogens.

To the authors' knowledge there is no published work indicating the presence of Avian Infectious *Laryngotracheitis* (AILT) in poultry in the country except a recent effort made by Hutton *et al*. (2017) to detect AILTV using PCR in chicken breeder farm in the central highlands of Ethiopia which was not successful. The present study therefore, describes the first outbreak of AILT in exotic layer chicken managed under semi-intensive production systems in Ethiopia. The outbreak was investigated based on the characteristic clinical signs, postmortem lesions and the pathognomonic microscopic lesions.

Materials and Method

Description of the outbreak and flocks affected

The outbreaks were observed in three poultry flocks located at Dore Bafano kebele

in Hawassa Zuria district in Sidam Zone of the Southern Nations, Nationalities and Peoples Regional State (SNNPRS) between June 10, 2018 and July 23, 2018. The farmers purchased the chicken, which were all Bovans brown, at about 50 days of age from different small scale poultry farms run by cooperatives. The later operate by receiving a day old chicks from a government owned large poultry farm center located at Hawassa city. The chickens were vaccinated for Newcastle disease (HB1 at day 7 and Lasota at day 21 and 42) and Gumboro disease (IBDV D78 vaccine at day 10 and 17) but they were not vaccinated for AILT. The health and production related issues of the flocks were regularly monitored by the local field veterinarian.

Each farm had 350 chickens on average and the chicken were managed in a poorly ventilated houses bedded with saw dust and wood shavings. During the day time, they were allowed to roam in the compound. During our visit to the farms we had the opportunity to see finches, doves and other wild birds sharing their feed and water. Moreover, clots of blood coughed up from critically sick chicken were seen attached on the floor, farm equipment and wall of the houses.

For further examination, a total of eight layer chickens with critical respiratory signs and four recently died chickens were collected and transported to the Veterinary Pathology laboratory of the Faculty of Veterinary Medicine at Hawassa University.

Necropsy and histopathological examination

All the collected chickens, both recently died and critically sick, were dissected for necropsy examination as per the standard procedure (Butcher and Miles, 2015). The gross lesions were characterized and recorded properly. Almost all sick and dead birds were in good body condition, suggesting that asphyxiation and hypoxia could be the major reason for death.

Histopathologic examination

Tissue samples from *trachea* with lesion were obtained and fixed in 10% neutral buffered formalin for 48 hrs. Later, the samples were processed by the paraffin technique, cut into 4–6µm thickness and stained with hematoxylin and eosin stain (H&E) and examined microscopically following the procedure recommended by Bancroft and Gamble (2008).

Result

Based on history and clinical presentation, the chickens were eight months old and showing extension of the neck, long-drawn-out gasps in an attempt to inhale (Figure 1), inappetence and periorbital swelling. Some chickens died after 5 to 8 days of illness. Some

Figure 1: Extension of the neck, long-drawn-out gasps in an attempt to inhale

Figure 2: Foamy lacrimation and blood tinged expectoration hanging on the beak of critically sick cock.

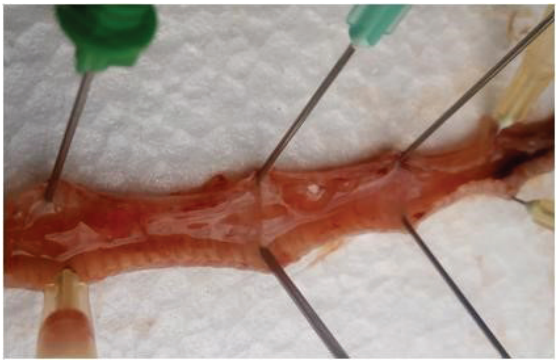

Figure 3: *Laryngotracheitis* with blood tinged mucus plugs in the lumen of critically sick chicken

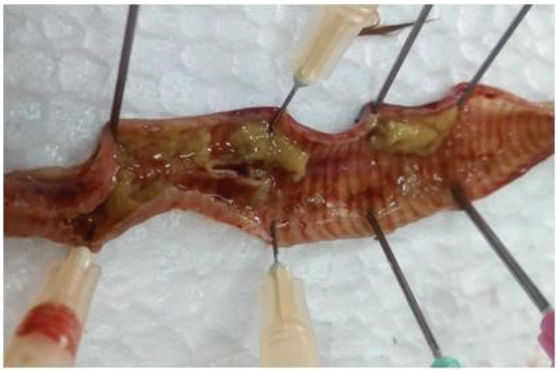

Figure 4: Abundant caseous/ fibrinous exudate accumulated in the lumen of *trachea* of recently died chicken

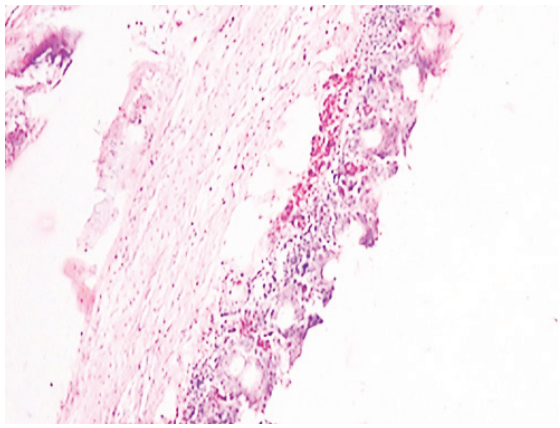

Figure 5: Erosion and ulceration of the *mucosa* with epithelial syncytia and mild to moderate hemorrhage. Edema and *lymphohistocytic* and heterophilic inflammatory infiltrates in *tracheal* sub*mucosa*, H&E, Obj.10x.

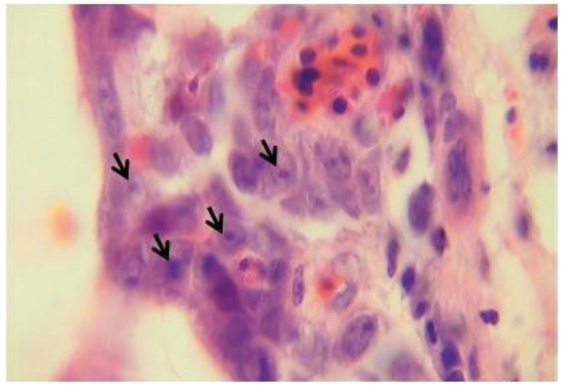

Figure 6: Hemorrhage, heterophils and syncytial cells with characteristic intranuclear inclusion bodies (arrow) in *tracheal* epithelial cells of acutely affected chickens, H&E, Obj.100x.

critically affected chicken exhibited a wide-open beak with repeated high-pitched squawk and foamy lacrimation (Figure 2). There was also gurgling, rattling and coughing when birds try to expel obstructions in the *trachea*. The mortality rate was about 80%. The morbidity was 98% and the mortality reached pick within two days of onset and then gradually declined but continued for 2 weeks. The prognosis was very poor even after Sulfa drug (*Sulphadimethoxine*, 0.5ml/day, IM) and Oxy tetracycline (200 g per 1,000 liters of drinking water during 3 – 7 days) administration.

The post-mortem lesions in all the critically sick chickens were totally confined to the upper respiratory tract (the sinus, *larynx* and the entire *trachea*) and were characteristic of AILT, consisting of hemorrhagic tracheitis with blood clots, mucoid rhinitis, and blood-stained mucus along the length of the *trachea* (Figure 3). Moreover, the post-mortem examination of the four recently died birds revealed diphtheritic or caseous necrotic plaques and plugs in the *trachea*, *larynx* and mouth (Figure 4).

Histopathologically, the lesions in *trachea* were characterized by erosion and ulceration of the *mucosa* with epithelial syncytia and mild to moderate hemorrhage. There was edema and *lymphohistocytic* and heterophilic inflammatory infiltrates in *tracheal* sub*mucosa* (Figure 5). Moreover, eosinophilic intranuclear inclusion bodies were observed in most of the *tracheal*

epithelial cells of the critically sick chickens, primarily in those forming syncytia (Figure 6).

Discussion

Avian Infectious *Laryngotracheitis* (AILT) is a highly contagious respiratory disease of chickens caused by a pneumotropic virus of the family Herpesviridae, genus Iltovirus called gallid herpesvirus 1 (GaHV-1) (Guy and Garcia, 2008; Parra *et al*., 2016). Although virus isolation and polymerase chain reaction (PCR) are mentioned as more sensitive tests for AILT (Guy *et al*., 1992; Williams *et al*., 1994; Abbas *et al*., 1996; Parra *et al*., 2016), histopathology, which can detect suggestive lesion, such as syncytia and intranuclear inclusion bodies, is the most common and relatively fast technique (Crespo *et al*., 2007). Moreover, in OIE terrestrial manual (2014), histopathology is recommended as a suitable method for confirmation of clinical cases of AILT. The present study, which was based on clinical, necropsy and histopathological examinations, reports for the first time the existence of AILT in chickens in Ethiopia. The clinical signs and, gross and histopathological lesions observed in the chickens affected by the outbreak were consistent with what has been reported by other studies and strongly suggestive of AILT. According to most previous reports (Beach, 1926; Bagust *et al*., 2000; Guy and Garcia 2008), the clinical signs observed, particularly nasal discharge, moist rales, coughing, gasping, dyspnoea, expectoration of blood-stained mucus with high mortality, are characteristic for AILT particularly the severe epizootic form. Moreover, the gross and histopathologic lesions (i.e. hemorrhagic tracheitis with blood clots and the presence of eosinophilic intranuclear inclusion bodies in *trachea*l epithelial cells and syncytia) observed in this outbreak were most striking and diagnostic. These lesions are widely accepted as pathognomonic for AILT (Purcell, 1971; Montgomery *et al*., 2007; Preis *et al*., 2013; Parra *et al*., 2016).

The outbreak was first observed in one flock and later, within few weeks of time, it was detected in two nearby poultry flocks probably due to spread from the index farm. Discussion with the responsible personnel of these farms revealed that they had neither purchased birds nor shared any material from the index farm. Therefore, airborne transmission via contaminated dust is thought to be the most likely means for the spread. It could also be by humans, rodents, wild birds and freely roaming dog and cats (Kingsbury and Jungherr, 1958; Johnson *et al*., 2005).

The source of AILT virus in the present outbreak was not clearly known, but presumed to be the presence of latent carrier birds in flocks or wild birds entering the poultry premises. It is well established that after natural infection or vaccination, the virus can remain latent in the trigeminal nerve of the *trachea*l sub*mucosa* throughout the life of the animal and virus reactivation and excretion occurs when birds are subjected to stress, such as the beginning of the laying period or when are mixed with unknown birds (Hughes *et al*., 1991; Coppo *et al*., 2013). Several epidemics in the world and introduction of the disease to a country previously free from the disease have been traced to the transport of birds in contaminated crates (Dufour-Zavala, 2008). Most intensive commercial farms in Ethiopia, including public poultry multiplication and distribution centers, import day old chicks from Egypt, Germany, Holland, Kenya, South Africa and UK (Alemu *et al*., 2008). Therefore, the virus/disease might have entered to the country through contaminated crates and remain undetected or misinterpreted because of the poor veterinary service and reporting systems. Moreover, most AILT outbreaks reported across the world were also associated with the use of live attenuated vaccines (Menendez *et al*., 2014). With the growing numbers of large private poultry farms and the poor legal restrictions on import, there is a suspicion that live attenuated vaccine might have been illegally imported and used in the country. If that is the case, live attenuated vaccines can establish latent infections and cause disease when allowed to spread from bird to bird (Bagust *et al*., 2000).

Conclusions

This study describes the first outbreak of AILT in chicken flocks in Ethiopia. Unless urgent control and preventive measure are taken, the disease can easily spread to other parts of the country because of unrestricted transport and trading of live birds with latent infections. Therefore, poultry producers and field veterinarians in different parts of the country should be aware of the disease and reinforce strict biosecurity measures to prevent the entry of the virus to their farm. Moreover, we recommend poultry farmers in the area to strictly bury or incinerate chicken died of unknown causes. As the present diagnosis was based on gross and histopathological examination, further studies using virus isolation and molecular techniques (like PCR) are required to confirm the disease.

Acknowledgment

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SONOGRAPHIC FEATURES OF GESTATION IN THE DEVELOPING GREATER CANE RAT (*THRYONOMYS SWINDERIANUS*)

Mustapha O A[1,4], Ajadi T A[2], Mustapha O O[3], Olaolorun F A[4], Popoola E S[1], Olude M A[1] and Olopade J O[4]

[1]Department of Veterinary Anatomy, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Ogun State, Nigeria

[2]Department of Veterinary Reproduction and Public Health, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

[3]Department of Veterinary Surgery and Theriogenology, Faculty of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria.

[4]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

Abstract

The Greater Cane Rat (GCR), a wild *hystricomorph* rodent found currently only in Africa, is widely hunted for meat and other economic benefits. This has led to its depletion in the ecosystem and has increased the drive for its domestication. However, there is a paucity of information on its reproductive biology. The traditional method of pregnancy diagnosis with digital abdominal palpation has been commonly reported and there is no record of the use of ultrasound detection of pregnancy in these rats. This study therefore described sonographic features of gestation in the GCR. Eleven pregnant greater cane rats (GCR), gestational ages ranging between 10 – 130 days, underwent abdominal ultrasonography using a portable ultrasound machine. The GCR were examined in dorsal recumbency following parenteral injection of 2mg/kg of *Xylazine* and 10mg/kg of *Ketamine*. The gestational sac, embryonal sac, crown rump length, bi-parietal diameter, foetal length and foetal diameter were measured. The presence of foetal heartbeats and/or movements were also noted. Gestational sacs detected at day 20 post-mating were the earliest sonographic feature of pregnancy in GCR. Sonographic features of embryos were first detected at day 50 post-mating. Embryonal sac lengths ranged between 10mm and 14mm, while embryonal sac diameters ranged between 14 mm and 20mm. Foetal structures with recognizable foetal limbs and organs were observed from day 110 post mating. It was concluded that brightness mode ultrasound is reliable for early detection of pregnancy in greater cane rats. Measurements obtained from this study will serve as baseline sonographic parameters that would be useful in the determination of gestational length in these rodents.

Key words: Ultrasonography, Gestation, Pregnancy, Foetus, Grasscutter.

CARACTÉRISTIQUES SONOGRAPHIQUES DE LA GESTATION DE L'AULACODE (*THRYONOMYS SWINDERIANUS*) EN CROISSANCE

Résumé

Le grand aulacode, un rongeur hystricomorphe sauvage que l'on ne trouve actuellement qu'en Afrique, est largement chassé pour sa viande et d'autres avantages économiques. Cette chasse a conduit à son épuisement dans l'écosystème et a accru la motivation pour sa domestication. Cependant, les informations sur sa biologie reproductive sont rares. La méthode traditionnelle de diagnostic de la gravidité par palpation abdominale digitale a été couramment rapportée, et il n'y a aucune trace de l'utilisation de la détection échographique de la gravidité chez ces rats. Cette étude a donc décrit les caractéristiques échographiques de la gestation des aulacodes. Onze aulacodes gravides, d'âge gestationnel de 10 à 130 jours, ont été soumises à une échographie abdominale à l'aide d'une échographe portable. Les aulacodes ont été examinées en décubitus dorsal après injection parentérale de 2 mg / kg de xylazine et 10 mg / kg

*Corresponding author email: jkayodeolopade@yahoo.com

de kétamine. Le sac gestationnel, le sac embryonnaire, la longueur du croupion de la couronne, le diamètre biparíétal, la longueur fœtale et le diamètre fœtal ont été mesurés. La présence de battements cardiaques et / ou de mouvements fœtaux a également été notée. Les sacs gestationnels détectés au jour 20 après l'accouplement constituaient la première caractéristique échographique de la gestation des aulacodes. Les caractéristiques échographiques des embryons ont été détectées pour la première fois au jour 50 après l'accouplement. Les longueurs des sacs embryonnaires variaient entre 10 mm et 14 mm, tandis que leurs diamètres variaient entre 14 mm et 20 mm. Des structures fœtales avec des membres et organes fœtaux reconnaissables ont été observés à partir du 110ème jour après l'accouplement. Il a été conclu que l'échographie en mode luminosité est fiable pour la détection précoce de la gravidité chez les aulacodes. Les mesures obtenues à partir de cette étude serviront de paramètres échographiques de base qui seraient utiles pour la détermination de la longueur gestationnelle chez ces rongeurs.

Mots-clés : échographie, gestation, gravidité, fœtus, aulacode.

Introduction

The Greater Cane rat, GCR, (*Thryonomys swinderianus*), also known as the grasscutter, is a wild *hystricomorph* rodent found currently only in Africa. It is among several animals that are used locally and regionally for meat production (Mensah and Baptist, 1986; Adoun, 1992; Addo *et al*., 2002). It is reported to be the most preferred and most expensive micro-livestock in West Africa (Asibey and Addo, 2000). This high demand and the economic benefits from its sale have resulted in aggressive hunting with complete disregard for its conservation and the environment. Therefore, domestication of the GCR is being encouraged in West Africa to help address these problems. Progress has however been slow due to the paucity of information on its reproductive biology (Yeboah and Adamu, 1995).

The gestational length in GCR is 148-158 days (Addo *et al*., 2007). Pregnancy is manifested in GCR by a definite change in body weight four weeks after mating and by intermittent vaginal bleeding (Addo *et al*., 2007). Vaginal mucus plug formation is first observed on day 59 of gestation, and could be used for pregnancy diagnosis (Adu and Yeboah, 2000). Animals with unplugged vaginas at 105 days after mating are however considered as not pregnant. The traditional method of pregnancy diagnosis in GCR is by digital palpation of the abdomen. This is usually combined with the presence of the vaginal mucus plug (Addo *et al*., 2007). Accurate prediction of the date of parturition is clinically useful to prevent or minimize reproductive losses by timely intervention (Kim *et al*., 2007). For the GCR reported to have a relatively high rate of embryonic re-absorption and abortion, the accurate assessment of gestational age can assist in decision making in breeding practices (England and Russo, 2006). More so, progress in assisted reproductive techniques, such as estrus synchronization and embryo transfer, requires accurate prediction of ovulation, gestational age, and parturition date (Tsutsui *et al*., 2006).

Ultrasonography is a safe and accurate modality for pregnancy diagnosis and no risks to either the operator or patient have been reported to date (Blanco *et al*., 2008; Kustritz, 2005). Three types of diagnostic ultrasound are described in the veterinary literature for canine pregnancy diagnosis. Amplitude depth ultrasound (A- mode) identifies the presence of fluid in and around the foetus. It cannot define the origin of the fluid as definitively uterine, nor does it allow assessment of foetal viability or number (England *et al*., 2003). Similarly, Doppler ultrasound provides an audible signal identifying foetal heartbeats, but gives no idea of foetal numbers or more exact information as to foetal viability (Blanco *et al*., 2008; Di Salvo *et al*., 2006). For these reasons, these two techniques are rarely used in rabbits. Brightness mode (B-mode), or real time ultrasound however, allows assessments of the pregnancy status, foetal numbers and viability, and investigation of the uterus and extra-reproductive abdominal structures (Kutzler *et al*., 2003). B-mode ultrasonography has been reported to be 94–98% accurate for pregnancy diagnosis

when used after 14–21 days of gestation, and 99% accurate for pregnancy diagnosis at greater than 22 days from the last breeding in rabbits (Gutierrez and Zamora, 2004). Foetal heartbeats have been noted from 15 days of gestation, while foetal movement has been reported to be visible from 12 days of gestation (Gutierrez and Zamora, 2004). Measurement of bi-parietal head diameter of foetuses, with or without measurement of dorso-ventral trunk diameter, has been demonstrated to be accurate for the estimation of gestational age in dogs and rabbits (Ajadi *et al*., 2015; Beccaglia and Luvoni, 2006).

Although the sonographic features of pregnancy in other *hystricomorph*s such as agouti and the guinea pig have been reported (Sekulic *et al*., 2009; Sousa *et al*., 2012), the use of ultrasound in the confirmation of pregnancy, determination of features of fetal development, or correlation with gestational age has not been defined. The study was therefore designed to determine the sonographic features and measurements at different stages of gestation in the GCR.

Materials and Methods

Animals and Housing

Eleven pregnant GCR at different stages of gestation, with a mean age (1.2 ± 0.5years) and mean body weight of 3.2 ± 0.4kg were used. They were obtained from a commercial farm (Onileola Farms®, Osun State, South West, Nigeria). The stages of gestation ranged from 10 days to 130 days post- mating. The animals were housed in hutches with a colony comprising of four females and one male. They were fed with grasses, cassava and sugar cane, while water was provided *ad-libitum*. They were maintained on cycles of twelve hours of day light and darkness prior to commencement of the study. The rats were allowed to mate naturally and successfully mated rats were temporarily kept in metal cages for the ultrasound examination.

Ultrasound Examination

Each rat was examined sonographically using a portable ultrasound machine with a 10.0 MHz transducer (Kaixin KX 2000R, GmbH, Ellfestrass, Hamburg, Germany). The machine was fitted with 3.5MHz curvilinear and 7.0MHz linear transducers. Each transducer has four windows of frequency range. Prior to examination, the GCR were anaesthetized with parental injection of 2mg/kg of 2% *Xylazine* Hydrochloride (*Xylazine* 20 Inj®, Kepro, Holland) and 10mg/kg of 5% *Ketamine* hydrochloride (Ketanir®, Kepro, Holland). Basic preparations including clipping of the hairs on the ventral abdomen and application of acoustic gel were performed. Once an appropriate image and position was obtained; it was paused for the measurements of the gestational sac, embryonal sac, crown-rump length, bi-parietal diameter, foetal length and foetal diameter. In addition, the presence of foetal heart beats and/or movements were noted.

Ethical approval for this work was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) with reference number UI-ACUREC/17/0066.

Results

The sonographic features at different stages of gestation in GCR are shown in Table 1. The earliest detectable sonographic feature of pregnancy in the GCR was the gestational sac. It was first detected by day 20 post mating and was characterized by an oval-shaped anaechoic sac containing a bipolar hyperechoic band (Figure 1). The gestational sac was observed also at day 40 post mating (Figure 2). Sonographic features of the embryo were first detected by day 50 post mating and were characterized by an oval-shaped anaechoic structure bounded by a hyperechoic rim with a central hyperechoic structure (embryo) (Figures 3, 4 and 5). Distinct foetal structures without recognizable foetal limbs and organs were observed by day 100 post mating (Figure 6), while foetal structures with recognizable foetal limbs and organs were observed from day 110 post mating up to day 130 post mating (Figures 7, 8 and 9).

Table 1: Sonographic features of gestation in Greater Cane Rat.

Days post mating	Observable sonographic features
10	No detectable sonographic features of gestation
20	Gestational sac (bi-polar hyperechoic structure) measuring 2mm in diameter
40	Gestational sac (bi-polar hyperechoic structure) measuring about 6mm in diameter
50	Embryonal sac represented as an oval shaped anaechoic structure bounded by hyperechoic rim with a central hyperechoic structure (embryo)
70	Embryonal sac represented as an oval shaped anaechoic structure bounded by hyperechoic rim with a central hyperechoic structure (embryo)
80	Embryonal sac represented as an oval shaped anaechoic structure bounded by hyperechoic rim with a central hyperechoic structure (embryo)
100	Foetal sac observed with distinct foetal structure without recognizable limbs and organs
110	Distinct foetal structures with recognizable foetal limbs and organs
120	Distinct foetal structures with recognizable foetal limbs and organs
130	Distinct foetal structures with recognizable foetal limbs and organs

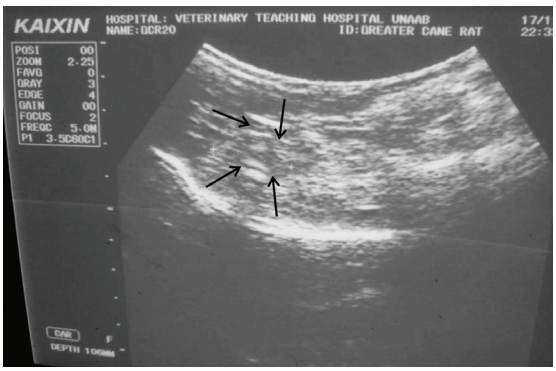


Figure 1: B-mode abdominal ultrasound of GCR at day 20 post mating showing gestational sac (arrows)

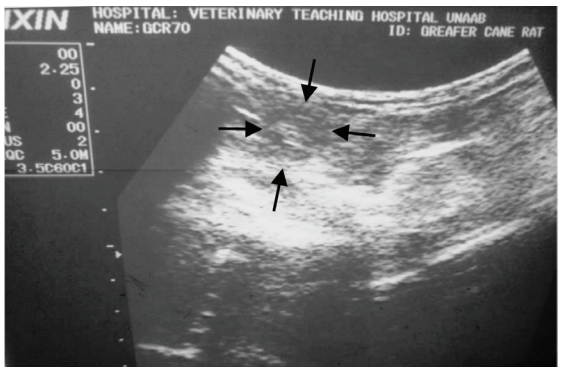


Figure 4: B-mode abdominal ultrasound of GCR at day 70 post mating showing embryonal sac (arrows)

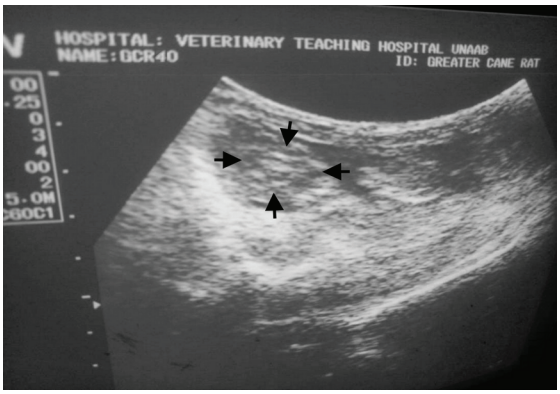


Figure 2: B-mode abdominal ultrasound of GCR at day 40 post mating showing gestational sac (arrows)

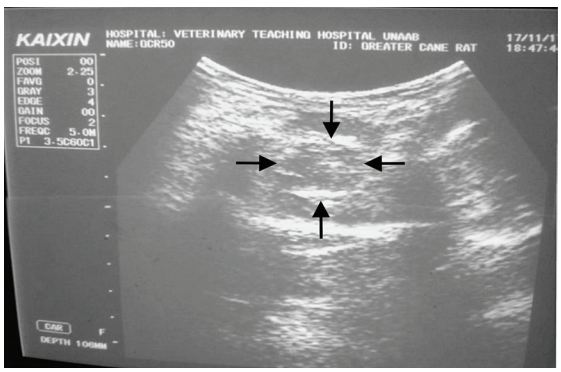


Figure 3: B-mode abdominal ultrasound of GCR at day 50 post mating showing embryonal sac (arrows)

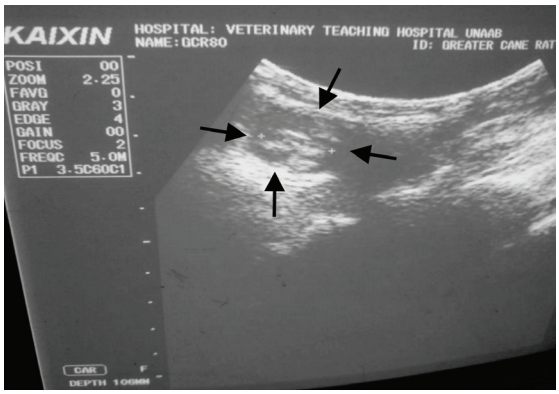


Figure 5: B-mode abdominal ultrasound of GCR at day 80 post mating showing embryonal sac (arrows)

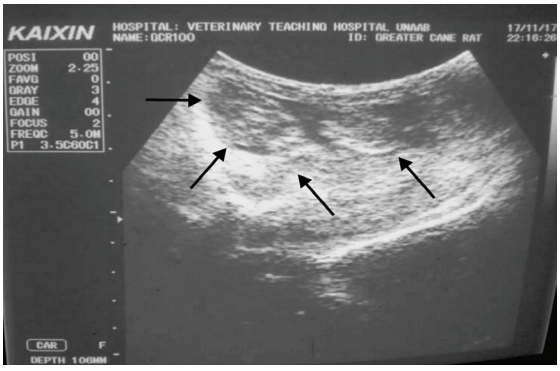
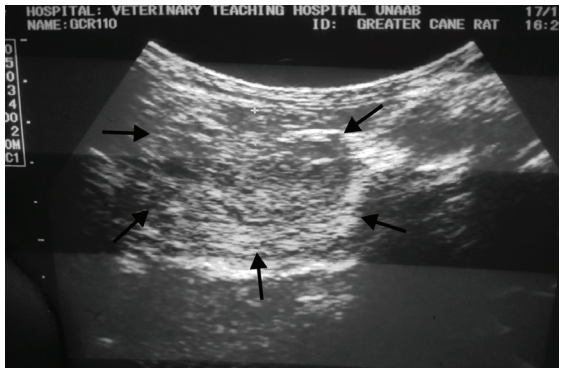


Figure 6: B-mode abdominal ultrasound of GCR at day 100 post mating showing foetal sac (arrows)



FiguVre 7: B-mode abdominal ultrasound of GCR at day 110 post mating showing a foetus (arrows)

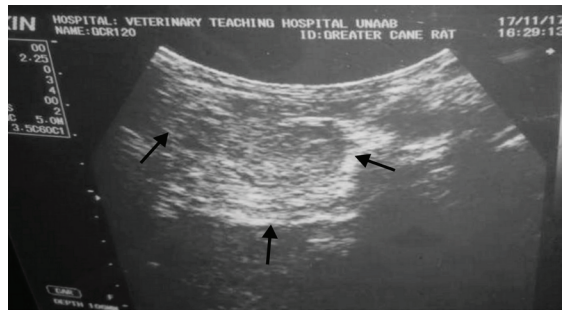


Figure 8: B-mode abdominal ultrasound of GCR at day 120 post mating showing a foetus (arrows)

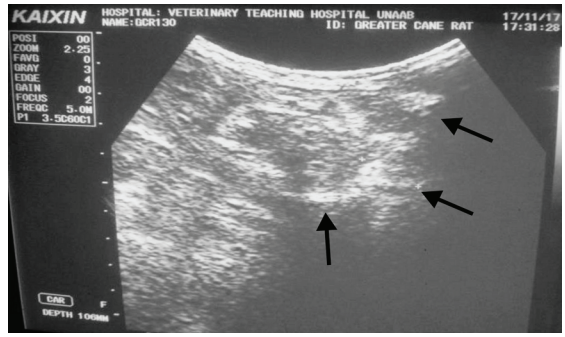


Figure 9: B-mode abdominal ultrasound of GCR at day 130 post mating showing a foetus (arrows)

Table 2: Sonographic measurements of gestational and embryonal sacs at different stages of gestations of Greater Cane Rat.

Days post mating	Gestational Sac Length (GSL)	Gestational Sac Diameter (GSD)
20	2mm	4mm
40	4mm	6mm
50	10mm	14mm
70	12mm	18mm
80	14mm	20mm

Table 3: Sonographic determination of Bi-Parietal Diameter (BPD), Crown Rump Length (CRL), Foetal Length (FL) and Foetal Width (FW) at different stages of gestation in Greater Cane Rat.

Days post-mating	CRL(mm)	BPD(mm)	FL(mm)	FW(mm)
100	36.0	14.0	30.0	17.0
110	43.0	22.0	43.0	25.0
120	48.0	25.0	49.0	25.0
130	56.0	29.0	50.0	25.0

The sonographic measurements at different stages of gestation in GCR are shown in Table 2. The gestational sac at day 20 post mating was 2mm in length and 4mm in diameter, while at day 40 post mating the gestational sac length was 4mm and the diameter was 6mm. The embryonal sac length was 10mm, while the embryonal sac diameter was 14mm by day 50 post mating. The embryonal sac length ranged between 10mm and 14mm, while the embryonal sac diameter ranged between 14 mm and 20mm. The sonographic dimensions of GCR fetuses at different stages of gestation are shown in Table 3. The crown rump length ranged between 36 mm and 56mm. The bi-parietal diameter ranged between 14mm and 29mm , while the foetal length ranged from 30mm to 50mm and the foetal width ranged between 17mm and 25mm.

Discussion

This is the first study of the sonographic evaluation of pregnancy in the GCR. In this study, the gestational sac was the earliest observable sonographic evidence of pregnancy in the GCR and was observed by day 20 post-mating. Other features observed included the embryonal sac from day 50 and foetal skeletal structures from day 100 post-mating. This finding varied from that reported for agouti in which the gestational sac was first detected by day 14, the embryonal sac by day 20 and the fetus was detected by day 55 (Sousa *et al*., 2012). The differences may be due to the longer gestational length in GCR compared with the 104 days reported for agouti and 59-72 days reported in guinea pigs.

Adequate restraint is required for proper sonographic evaluation during pregnancy in the GCR. Movement of the animal can result in artifacts and affect the quality of the image. Owing to the wild nature of GCR and the difficulty in applying physical restraints alone, they were anaesthetized with a combination of low doses of *Xylazine* and *Ketamine*. These two drugs are potent sedative hypnotics and are known to cross the placenta to depress the foetus (Janssen *et al*., 2004; Sumitra *et al*., 2004). However; the use of a *Ketamine*-diazepam combination for the chemical restraint of pregnant GCR for sonographic evaluation would be a safer alternative (Sumitra *et al*., 2004).

Part of the routine skin preparation for ultrasound examination is the shaving of the hair before application of ultrasound gel. The skin of the GCR is covered with individual thick spiny hairs which are sharply pointed and pliable (Skinner and Chimimba, 2005). Thus, it is important to shave the hair on the ventral abdomen before gel application in order to ensure excellent image acquisition. Chemical restraint is necessary for the preparation process as the skin is delicate and easily gets stripped.

This study showed that the gestational sac was the earliest recognizable sonographic feature in pregnant GCR and was observed by day 20 after mating. This finding is similar to that observed in the agouti in which the gestational sac was detected between day 14 and 20 (Sousa *et al*., 2012). The sonographic features of the gestational sac in the GCR were similar to those reported for dogs (Luvoni and Grioni, 2000), rabbits (Ajadi *et al*., 2015) and agouti (Sousa *et al*., 2012).

The embryonal sac was first recognized at day 50 post mating. The embryonal sacs were easier to recognize sonographically than the gestational sacs because they were bigger in size and more anaechoic. Although pregnancy can be detected as early as day 20 post mating in GCR, a confirmatory diagnosis can better be made around day 60 post mating when both the presence of the vaginal mucus plug and a more easily detectable embryonal sac can be used.

Recognition of the foetal heart beat and foetal movement is useful in the assessment of foetal viability and to detect foetuses that are stressed. In the agouti, the fetal heart beat was detected by day 25 post-mating, (Sousa *et al*., 2012). However, foetal heart beat and foetal movement were not detected in this study. This may probably be due to the agents used to chemically restrain the GCR. Both *Xylazine* and *Ketamine* have been reported to cross the placenta to cause foetal depression, although, *Ketamine* is reported to produce less depression than *Xylazine* (Sumitra *et al*., 2004). In the sonographic detection of pregnancy in agouti, the animals were neither sedated nor anaesthetized.

Conclusion

The result of this study confirms the reliability of B- mode ultrasound in the detection of pregnancy in the GCR. Ultrasound examination can detect pregnancy as early as 20 days post-mating, much earlier than the traditional detection of the vaginal mucus plug. However, it is better to perform detection of pregnancy around day 60 post-mating when sonographic detection of the embryonal sac and the presence of a vaginal plug can be better used to confirm pregnancy. The sonographic measurements obtained in this study will serve as baseline sonographic measurements during gestation in GCR. This will be useful in breeding management and for future experimental studies in the fields of reproductive and developmental biology in this rodent.

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SIZE OF BEESWAX FOUNDATION SHEET AND PRODUCTIVITY OF HONEYBEE COLONIES IN NORTHEASTERN ETHIOPIA

*Tewodros Alemu[1] and Muluken Girma[1]
[1]Wollo University, College of Agriculture, Department of Animal Science, P.o.box: 1145, Dessie Campus, Dessie, Ethiopia

Abstract

The study was conducted in Ambasel district (Ethiopia) to determine the effect of the size of beeswax foundation sheet on the productivity and profitability of frame hive beekeeping. Comparatively similar population sizes of twenty colonies were selected and randomly assigned to five levels of treatments [100%, 75%, 50%, 25% and 0% (smear) of wax sheet]. The experimental design used was CRD. Since the initial colony population difference became obligatory, the variability was captured through covariance analysis using the initial colony size difference as a covariate. The number of adult & brood populations of each hives was estimated using Liebefelder method. Data on honey yield, brood and adult populations were analyzed using SAS and to compare the profitability among the treatments SPSS was used. For partial budget analysis the costs of beeswax, labour, wire and fuel (for beeswax melting) were used. Full foundation sheet (100%) was significantly higher ($p<0.05$) in honey yield than the other treatments. Smear (0%) was significantly higher ($p<0.05$) in honey yield as compared to treatments 50% and 25%. On the other hand, smear was higher in wax yield than the other treatments which might be due to stimulation of honeybees to secret wax. The net benefit obtained from smear was higher followed by 100%. Generally the net benefit obtained from the treatments was low because the experimental colonies were attacked by varoa mite during the study period. For more reliable information, it is recommended to collect data for two or more honey flow periods.

Key words: honeybees, beeswax, size, productivity

TAILLE DE LA FEUILLE DE FONDATION DE CIRE D'ABEILLE ET PRODUCTIVITÉ DES COLONIES D'ABEILLES MELLIFÈRES DANS LE NORD-EST DE L'ÉTHIOPIE

Résumé

L'étude a été réalisée dans le district d'Ambasel (Éthiopie) dans l'objectif de déterminer l'effet de la taille de la feuille de fondation de cire d'abeille sur la productivité et la rentabilité de l'apiculture à cadres et à ruches. Des tailles de populations relativement similaires de vingt colonies ont été sélectionnées et assignées de manière aléatoire à cinq niveaux de traitements [100%, 75%, 50%, 25% et 0% (enduit) de feuille de cire]. L'étude a utilisé la conception expérimentale CRD. Étant donné que la différence initiale entre populations des colonies est devenue obligatoire, la variabilité a été mise en évidence par une analyse de covariance utilisant la différence de taille initiale de colonie comme co-variable. Le nombre de populations d'adultes et de couvains de chaque ruche a été estimé au moyen de la méthode de Liebefelder. Les données sur le rendement en miel, les couvains et les populations adultes ont été analysées à l'aide de la méthode SAS, et pour comparer la rentabilité des traitements, la méthode SPSS a été utilisée. Pour une analyse budgétaire partielle, les coûts de la cire d'abeille, de la main-d'œuvre, du fil et du carburant (pour la fusion de la cire d'abeille) ont été utilisés. La feuille de fondation complète (100%) avait un rendement en miel significativement plus élevée ($p <0,05$) par rapport aux autres traitements. L'enduit (0%) avait un rendement en miel significativement plus élevée ($p <0,05$) par rapport aux traitements à 50% et à 25%. En revanche, l'enduit avait un rendement plus élevé en cire par rapport aux autres traitements, ce qui pourrait être dû à la stimulation des abeilles à la cire secrète. Le bénéfice net obtenu à partir de l'enduit était plus élevé, suivi du traitement à 100%. Généralement, le bénéfice net obtenu à partir des traitements était faible

*Corresponding author email: Tewodros Alemu, E-mail: teda2010@gmail.com

car les colonies expérimentales ont été attaquées par des acariens Varoa pendant la période d'étude. Pour des informations plus fiables, il est recommandé de collecter des données de deux ou plusieurs miellées.

Mots-clés : Abeilles mellifères, cire d'abeille, taille, productivité

Introduction

Ethiopia is one of the few countries in the world with a long tradition of beekeeping that gave an opportunity of supplying honey and beeswax to the international markets. The ancient tradition of beekeeping in Ethiopia stretches back into the millennia of the country's early history. According to Hartmann (2004) of all countries in the world, probably no country has a longer tradition of beekeeping than Ethiopia. This could be attributed to Ethiopia's wide climatic and edaphic variability which have endowed the country with diverse and unique flowering plants, thus making it highly suitable for sustaining a large number of honeybee colonies and the long established practice of beekeeping (Girma, 1998). Although Zander hives were recently introduced in the country, modern beekeeping method using Zander hive have attracted the attention of most beekeepers (Tewodros *et al*., 2017).

The country is estimated to have ten million honeybee colonies, which is the largest in Africa. This makes Ethiopia the leading producer of honey and beeswax in Africa and 10th in honey production in the world (SOS-Sahel, 2006). The country is one of the 3rd largest beeswax producing countries in the world next to China and Mexico (ARD, 2007) and she ranked 4th in beeswax export. The most important honey and beeswax producing regions in Ethiopia are Oromia (contributing 36 % of total production), SNNPR (31%), Amhara (19%), Tigray (5%) and other regions (9%) (EPPA, 2003).

Currently based on the level of technological advancement, three types of beehives are used for honey production in Ethiopia. These are traditional, intermediate, and modern hives. A total of about 4,601,806 hives exist in the country of which about 95.5% are traditional, 4.3% transitional and 0.20% modern hives (Beyene and David, 2007).

Beeswax is widely used as a waterproofing agent, candle making, as an ingredient in ointments/lubricants, medicines, soaps and polishes, in the manufacture of electronic components and CDs, in modeling and casting, in grafting and in artificial comb formation. The most important use of beeswax is in beekeeping itself, namely for the production of artificial combs. Artificial comb foundation is made of moulded or pressed wax sheets with cells imprinted on them that the bees very quickly and economically (using very little honey) build into comb. A surplus of beeswax can be found mainly in countries where artificial comb foundation is not used (Marieke *et al*., 2005). In frame hives of beekeeping, empty honeycombs are returned to the hive after the extraction of honey, which means that relatively little beeswax is harvested. With frame hives, the ratio of honey to beeswax production is approximately 75:1. However, the ratio of honey to beeswax production using traditional/top-bar hives is about 10:1 (Nicola Bradbear, 2004). For this reason, Ethiopia produces large amounts of beeswax, which provide sufficient inland consumption and a valuable export. Since the country is nowadays shifting from traditional to modern beekeeping, the amount of wax production will decline.

The bees sweat wax out of their glands to build their honeycomb nest. The building material and supply of energy for this activity is honey. While producing and building with wax, the bees eat and digest a lot of honey (Marieke *et al*., 2005). Therefore, in order to produce more honey as one means of ensuring food security as is placed in the GTP of the country, the sector should be transformed from traditional to modern/frame hives. However, the modern/frame hive is not without limitations. Among the shortcomings, it demands beeswax as a foundation sheet. Ethiopian beeswax export is about 620 tons/year (Amsalu *et al*., 2006). This is around 20% of the total beeswax production

in the country. With a simple calculation of 3kg beeswax for a single hive foundation sheet making, if the country is intended to transform additional 10% of the traditional hive in to modern hive, the work will demand more than 1300 tone of beeswax that is equivalent with around double of the current annual beeswax export or 40% of the national production.

Now days the country is facing severe shortage of beeswax for the use of foundation sheet in the program of improving the production and productivity of honey. This is due to wastage of large portion of wax (particularly at farm gates) due to lack of awareness of its market value (Beyene T. and David P., 2007), the relative expansion of frame hives and export of beeswax. As a result, the price of wax is escalating and currently the price of one kg of wax is on average more than 50% of the price of honey. The rise in price of beeswax leads to an adulteration with animal fats, petroleum, polyethylene, etc. and has become a great threat to the apiculture industry. These all-aforementioned limitations are calling for a wise utilization of the beeswax produced, the promotion of modern hives and the need for an appropriate technologies for so. Therefore the idea was initiated in an attempt to test some alternatives for the wise and economical utilization of beeswax to enhance and sustain honey production in the country so that the beekeeping sector will play its value in improving the livelihood of the community. The experiment was therefore designed to determine the effect of the size of beeswax foundation sheet on the productivity and profitability of frame hive beekeeping.

Materials and Methods

The Study Area

The research was conducted in collaboration with 'Tisabalima honey production and marketing private cooperative' which is located in Tisabalima kebele (lowest administration unit of Ethiopia), Ambasel district, Amhara region, Ethiopia.

The Treatments and the Experimental Design

Comparatively twenty similar in population size of colonies were selected and randomly assigned to five levels of treatments which are described below. The experimental design used was CRD. Since the initial colony population size differences became obligatory, the variability was caught through covariance analysis using the initial colony difference as a covariate. The treatments used in the study were: 100% level of foundation sheet (control) (120 gm); 75% level of bees wax foundation sheet (90 gm); 50% level of bees wax foundation sheet (60 gm); 25% level of bees wax foundation sheet (30 gm); and smearing (near to 0%).

Method of Data Collection

Formats were prepared to collect data on the number of adult bees and brood, to collect cost data related with production of honey. Prices of variable costs of the treatments such as beeswax and honey were recorded. The initial honeybee numbers of the colonies were estimated using Liebefeld Method (Anton Imdorf and Luzio Gerig, 2001). This method assumes the number of adult bees and brood per occupied side of zander hive comb is 1000 and 3200, respectively. A prototype frame was then prepared which has 8 cells per side with 10cm*10cm each cell. The prototype frame is for ease of estimation in which each cell will contain 125 bees or 400 broods. The total brood area was measured using a wooden frame and placed over each side of the brood combs. The total brood population was calculated from the total area occupied by the brood.

Honey yield data was recorded by weighing frames containing ripen honey before and subtracting from its initial weight. Honey data were collected by weighing the frames and taking the difference. The yield was expressed in Kg/colony.

Management of the honeybee colonies

Twenty honeybee colonies of traditional hives were purchased. The honeybee colonies were then transferred to modern hive. The type of modern hive used for each treatment was Zander. The colonies of traditional hives were

transferred to the Zander beehives during October 4 and 5/2014. The arrangement of the experimental honeybee colonies is shown below (Figure 2). The source of beeswax (tej breweries of Dessie town) was similar for each treatment.

Type of data collected and inputs used

The types of data collected were: Honey yield, Brood population, Adult population, Costs related with production and Income estimate which would be fetched from the honey sell. Inputs like honeybee colonies, frame hive, beeswax, beekeeping materials and equipment and weighing scale were also used.

Data analyses

Honey yield, brood population and adult population data were analyzed using general linear model procedure of the statistical analysis system (SAS). So as to attest the profitability of the different technologies partial budget analysis was done using SPSS statistical software. Mean separations were tested using least significance difference (LSD) at a significance level of 5%.

Figure 1: Adjustment of the frames and foundation sheet

Results

Rendering crude wax, printing and fixing the foundation sheet

The printed foundation sheet was measured and cut according to the treatments (Figure 1A). The frames of the modern hives were wired with frame wires to strengthen the fixture of the wax sheet with the frames (Figure 1C). David (2008) indicated wire is usually embedded in the sheet to hold it in the frames and to prevent damage. Moreover, the prepared foundation sheets according to the treatments were attached to the frames using wire pieces (Figure 1B).

Management of the honeybee colonies

The arrangement of the experimental honeybee colonies is shown below (Figure 2).

Figure 2: Arrangement of honeybee colonies of the experiment

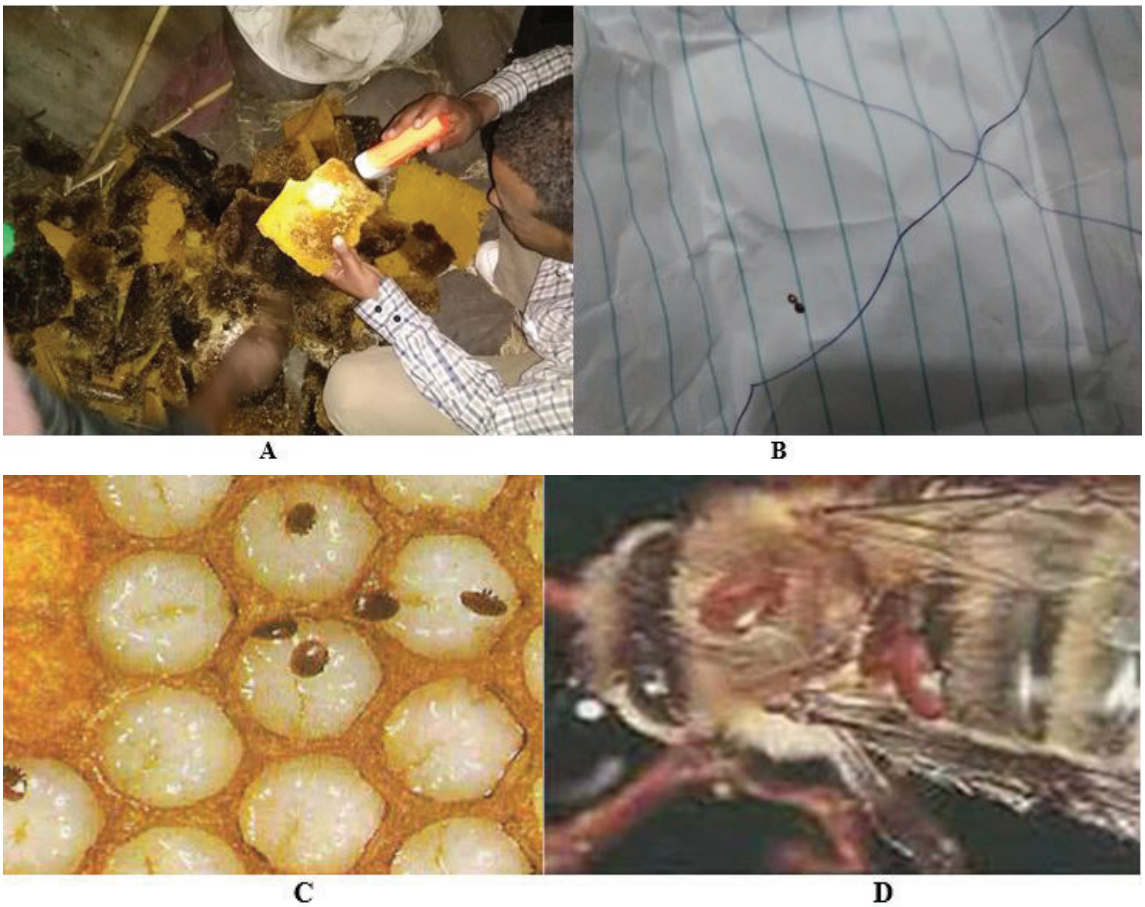

Figure 3: Infestation of the comb by varoa mite (A), the picture of the mite (B), Varroa mite on an adult bee (D) and Varroa on larvae (C)

Major problems encountered to the experimental honeybee colonies

The decline in the number of honeybees was caused mainly by heavy infestation of varoa mite. Moreover, there was a prevalence of wax moth, heavy cold and cloud starting (Figure A and B).

Estimation of initial honeybee colonies' size

As the hive is opened some bees were scattered to the lid and wall of the hive. These were also estimated accordingly. Both sealed and open broods were estimated. The initial honeybee numbers of the colonies are indicated in table 1. After three weeks colonies had growing the given foundation sheet and made brood (Figure 4 A and B).

Honey and wax yields of the experimental honeybee colonies

The mean honey and wax yields of the treatments are indicated in table 2.

Partial budget analysis

The partial budget analysis of the treatments is indicated in table 3. The current average price of beeswax (300 birr/kg) and honey (100 birr/kg) was used for the analysis.

Table 1: Initial colony size (adult bees and brood) of the treatments

Treatment	N	Number of adult bees	SE	Brood number	SE
0%	4	11038[a]	1412	9016[a]	3892
25%	4	5151[a]	1631	4414[a]	4489
50%	4	8925[a]	1412	6108[a]	3892
75%	4	9283[a]	1631	15629[a]	4489
100% (control)	4	6714[a]	1412	5600[a]	3892

Means with the same letter are not significantly different; SE = standard error

Figure 4: The growth of foundation sheet (grown from A to B)

Table 2: Mean honey and wax yield of the treatments

Treatment	N	Honey yield (kg)	SE	Wax yield (kg)	SE
100% (control)	4	8.75a	1.36	0.089a	0.04
75%	3	5.88ab	1.55	0.188a	0.048
50%	4	3.5ab	1.36	0.193a	0.04
25%	3	2.72b	1.55	0.198a	0.048
0%	4	6.62ab	1.36	0.6b	0.04

Means with the same letter are not significantly different; SE = standard error

Table 3: Partial budget analysis of the treatments in Ethiopian birr

Parameters	Treatments				
	0% (smear)	25%	50%	75%	100% (control)
Average Honey Yield (kg)	6.62	2.72	3.5	5.88	8.75
Average Wax Yield (kg)	0.6	0.198	0.193	0.188	0.089
Gross Field Benefit (birr)	843	294.3	407.8	609	901.6
Costs (birr)	19.73	220.75	407.75	591.75	772.5
Net Benefit	823.27[a]	73.65[c]	0.15[c]	17.25[c]	129.2[c]

Discussion

Wax sheet was prepared by purchasing crude wax from tej breweries of Dessie town. The purchased crude wax was rendered by hot water till the temperature rises above 65 °C, squeezed and purified using jute (Marieke, 2005). In this experiment, rendering of crude wax was preferred over purchasing readymade pure wax to show/train the cooperative farmers how crude wax is rendered from locally collected crude wax although it is not the objective of the research work.

The purified wax was then printed into foundation sheet using casting mould. The printed foundation sheet was measured and cut according to the treatments (Figure 1A). The frames of the modern hives were wired with frame wires to strengthen the fixture of the wax sheet with the frames (Figure 1C). David (2008) indicated wire is usually embedded in the sheet to hold it in the frames and to prevent damage. Moreover, the prepared foundation sheets according to the treatments were attached to the frames using wire pieces (Figure 1B).

The type of management used was the normal/usual practice of beekeepers and was similar to all the experimental treatments. Supering of the hives was carried out during October 30/2014. The time of supering was when outer frames have had their wax foundation drawn out as recommended by David Wootton (2010).

Initially during the time of colony transfer the colonies were strong. However, during the time of supering there was a sudden decline in the number of honeybees in each colony. The number of honeybees was continued to be dwindled. This condition was not only limited to the experimental colonies but also to all honeybee colonies of the surrounding area.

The decline in the number of honeybees was caused mainly by heavy infestation of varoa mite (Figure A and B). Moreover, there was a prevalence of wax moth, heavy cold and cloud. This was in line with the findings of Tewodros *et al*. (2015) who reported the major constraints for the development of apiculture were drought, pests and predators and application of chemicals. As a result, most of the colonies were not strong enough to be able to supered during the time of supering. Eight of the colonies were only able to be supered.

The initial honeybee numbers of the colonies were estimated using Liebefeld Method (Anton Imdorf and Luzio Gerig, 2001). This method assumes the number of bees and brood per occupied side of zander hive comb is 1000 and 3200, respectively. A prototype frame was then prepared which has 8 cells per side with 10cm*10cm each cell. The prototype frame is for ease of estimation in which each cell will contain 125 bees or 400 broods. As the hive is opened some bees were also scattered to the lid and wall of the hive. These were also estimated accordingly. Both sealed and open broods were estimated. The initial honeybee numbers of the colonies are indicated in table 1.

The data record on the estimation of the colony size was carried out in October 4 and 5 during the time of transferring honeybee

colonies from traditional to modern hives. The initial colony sizes were covariated during analysis to reduce the experimental error. After three weeks they have growing the given foundation sheet and made brood (Figure 4 A and B).

Treatment 1 (full foundation sheet) was significantly higher ($p< 0.05$) in honey yield than the treatment 25% (Table 2). The other treatments have no significant difference ($p> 0.05$) in honey yield among them. This might be due to the high infestation level prevailed in treatment 25% as compared to treatments 75%, 50% and 0%. Treatment 5 (smear) was significantly higher in wax yield than the other treatments (Table 2). This might be due to the stimulation of the honeybees to secret wax for storage of their honey.

The partial budget analysis was done based on the cost of beeswax, labour used, wire and fuel energy to melt the beeswax. Beehive, honey extractor, smoker and other appliances were used for all treatments equally. Two hives from 0% treatment, one hive from 25% treatment, one hive from 75% treatment and all of the 100% was supered. However there was no any hive supered from 50% treatment. The current average price of beeswax (300 birr/kg) and honey (100 birr/kg) was used for the analysis. The labour cost incurred was the cost for wax melting, printing the foundation sheet and fixing the wax sheet with the frames.

The partial budget analysis showed that the net benefit obtained from treatment 1 (smear or 0% wax sheet) was significantly higher than the other treatments. Generally the net benefit obtained from the treatments was very low due to varoa mite and wax moth attacks which made the colonies a severe decline in honey yield up to loss of the colonies via absconding (Table 3). So we recommend the experiment to be conducted for two or more honey flow seasons to have more reliable information.

Conclusion

Full foundation sheet (100%) was significantly higher in honey yield followed by smear (0%). On the other hand smear was higher in wax yield than the other treatments. This might be due to the stimulation of the honeybees to secret wax for storage of their honey. Great wax moth attack was observed in the colonies which could probably come from the traditional hive during transferring. At the end of the experiment the colonies was again attacked by varoa mite. The partial budget analysis showed that there the net benefit obtained from the treatment of smear or 0% was higher than the other treatments. Generally the net benefit obtained from the treatments was very low due to varoa mite and wax moth attacks which made the colonies a severe decline in honey yield up to loss of the colonies via absconding. So we recommend the experiment to be conducted for two or more honey flow seasons to have more reliable information.

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PHYSICAL CHARACTERISTICS, MICROBIAL CONTENTS AND CHEMICAL COMPOSITION OF SILAGES PRODUCED FROM IMPROVED NATURAL PASTURE WITH OR WITHOUT ADDITIVES

Ojo V O A[1]*, Olanite J A[1], Jolaosho A O[1], Muraina T O[2], Otaru E T[1] and Adeoye S A[1]
[1]Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta P. M. B. 2240, Abeokuta, Nigeria.
[2]Department of Animal Health and Production Technology, Oyo State College of Agriculture and Technology, P. M. B. 10, Igbo-Ora, Nigeria

Abstract

This experiment was conducted to evaluate the effects of tillage practices, sowing methods and additives on the physical, microbial and chemical composition of silages produced from *Stylosanthes* species oversown into natural pasture dominated by *Panicum maximum*. The factors in this study A 2 × 2 × 2 × 4 factorial arrangement which included two herbaceous legumes (*Stylosanthes guianensis* cv. Cook and *Stylosanthes hamata* cv. Verano), two tillage methods (zero and minimum tillage) and two sowing methods (broadcasting and drilling). Forage samples were harvested after nine (9) months of growth, chopped and wilted. The chopped forages was used for the statistical analysis were divided into four parts and ensiled in laboratory bottles (960 ml) silos with three additives (molasses, salt and molasses-salt) and a control. Silages were analyzed for microbial contents and chemical composition. The results showed that all silages made with molasses-salt additive had the best physical parameter scores. Silage made from a plot oversown with *Stylosanthes hamata* had higher (P>0.05) level of lactic acid bacteria. Silage made from forages harvested from minimally tilled soil recorded higher (P>0.05) crude protein content. The pH values of the silage irrespective of the factors were within the range for good quality silage. Silages made from a plot oversown with *Stylosanthes hamata* had higher (P>0.05) CP content than silage made from a plot oversown with *S. guianensis*. It can be concluded that silage that was made from natural grassland oversown with *S. hamata* with minimal tillage and molasses-salt as additive will provide a high quality feed resource for ruminants especially during the dry season when feed are scarce.

Keywords: Additive, legume, silage, nature pasture, oversown

CARACTÉRISTIQUES PHYSIQUES, CONTENU MICROBIEN ET COMPOSITION CHIMIQUE DES ENSILAGES PRODUITS À PARTIR DE PÂTURAGES NATURELS AMÉLIORÉS AVEC OU SANS ADDITIFS

Résumé

Cette expérience a été menée dans l'objectif d'évaluer les effets des pratiques de travail du sol, des méthodes de semis et des additifs sur la composition physique, microbienne et chimique des ensilages produits à partir d'espèces de *Stylosanthes* ensemencées dans des pâturages naturels dominés par *Panicum maximum*. Les facteurs utilisés dans cette étude comprenaient deux légumineuses herbacées (*Stylosanthes guianensis* cv. Cook et *Stylosanthes hamata* cv. Verano), deux méthodes de travail au sol (labour zéro et minimum) et deux méthodes de semis (le semis à la volée et le semis en lignes). Des échantillons du semis en lignés ont été récoltés après neuf (9) mois de croissance, hachés et flétris. Les fourrages hachés ont été divisés en quatre parties et ensilés dans des bouteilles de laboratoire (960 ml) en silos avec trois additifs (mélasse, sel et mélasse-sel) et un témoin. Les ensilages ont été analysés pour leur contenu microbien et composition chimique. Une analyse statistique a été réalisée en utilisant une analyse de variance à quatre voies. Les résultats ont montré que tous les ensilages ayant un additif mélasse-sel avaient les meilleurs scores des paramètres physiques. L'ensilage issu d'une parcelle recouverte de *Stylosanthes hamata* avait un

*Corresponding author email: ojovoa@funaab.edu.ng

niveau plus élevé (P> 0,05) de bactéries lactiques. L'ensilage obtenu à partir de fourrages récoltés dans un sol peu labouré a enregistré une teneur en protéines brutes plus élevée (P> 0,05). Les valeurs de pH de l'ensilage, quels que soient les facteurs, se situaient dans la plage d'un ensilage de bonne qualité. Les ensilages fabriqués à partir d'un terrain ensemencé avec *Stylosanthes hamata* avaient une teneur en PB plus élevée (P> 0,05) que l'ensilage fabriqué à partir d'un terrain ensemencé avec *S. guianensis*. On peut conclure que l'ensilage fabriqué à partir de pâturages naturels ensemencés avec *S. hamata* avec un labourage minimal et un additif de sel - mélasse fournira une ressource alimentaire de haute qualité pour les ruminants, en particulier pendant la saison sèche lorsque les aliments pour animaux sont rares.

Mots-clés : Additifs, légumineuse, ensilage, pâturage naturel, ensemencé

Introduction

Ruminants are prone to poor nutrition most especially during the dry season in tropical countries. During this period, animals are sustained mainly on unimproved natural pastures and crop residues which are highly lignified, containing low protein and essential minerals. Grazing animals on natural pastures during the dry season have led to poor growth, increased weight loss and sometimes high rates of mortality. The use of concentrate feeding during the dry season for ruminants has been recommended by various researchers (Little *et al*., 1991; Moss, 1993). However, dry season concentrate feeding of ruminants is uneconomical because of the high cost and sometimes the scarcity of ingredients required. There is a need therefore to explore possible ways of improving natural pastures so as to provide a high quality and quantity forage for animals during the dry season.

A possible way of improving natural pastures is through oversowing with improved and proven forage legumes such that the nutritive quality of the resulting feed resource from these natural pastures could be enhanced (Kusekwa and Lugenja, 1983).

In combating the poor quality of forages, especially during the dry season, the conservation and nutritive improvement of the tropical forages will ensure sustainable animal feeding and increase production. The use of various additives in silage production have been reported as a means of improving fermentation, enhancing the nutritive value and reducing storage losses of the resultant feed (Balieiro Neto *et al*. (2007). Among the most commonly used additives are molasses and common salt (NaCl). Molasses has been widely used to provide fast fermentable carbohydrate for the ensilage of tropical herbages (Yitbarek and Tamir, 2014). Moreover, due to its strong activity on food micro-organisms, ability to alter pH levels and reduce water activity, NaCl has been extensively used in the fermentation process and silage production (Rabelo *et al*., 2013).

Provision of silage is based on anaerobic fermentation, where it is controlled primarily by the type of micro- organisms that dominate the fermentation and play an important role in the successful outcome of the conservation process. Epiphytic lactic acid bacteria convert water-soluble carbohydrates into organic acids, mainly lactic acid (Weinberg *et al*., 2010). As a result, a low pH value inhibits the undesirable microorganisms and the forage crop is preserved. Also, chemical analysis coupled with visual examination, such as colour, odour and general appearance provide a good indication of the expected overall nutritive value of silage.

This study was conducted to evaluate the physical characteristics, microbial contents and chemical composition of silages produced from legumes oversown into natural pasture in Southwestern Nigeria.

Materials and Methods

Location and climate of the study area:

The research was carried out at in a town located in Yewa North Local Government Area of Ogun State, South West Nigeria. The experimental site lies within latitude 7° 1° 2.6" N, longitude 2° 50° 41"E at altitude 13447 ft (Google Earth, 2013). The research site is in the Derived Savannah agro-ecological zone

of South western, Nigeria and has an average annual rainfall of 1230 mm with a bimodal rainfall pattern which peaks in July and September. It experiences high (63-96%) and low (55-84%) relative humidity during the rainy and dry seasons respectively. Mean monthly temperatures rangd from 25.70°C in July and 30.20°C in February.

Experimental design:

The experiment was laid out as a 2 × 2 × 2 × 4 factorial arrangement with two herbaceous legumes (*Stylosanthes guianensis* cv. Cook and *Stylosanthes hamata* cv. Verano), two field tillage operations (zero and minimum tillage (15-cm depth)), two planting methods (broadcasting and drilling) and three types of silage additives (molasses (4%), salt (0.5%), molasses (2%) + salt (0.25%) and a control). The study was replicated four times with each plot measuring 3 m^2.

Sowing of herbaceous legumes, silage preparation and chemical composition:

The *Stylosanthes* species which were scarified in hot water of 80°C for 3 minutes were oversown into zero- and minimally-tilled natural pasture that was dominated by *Panicum maximum* in September, 2013. Soil analysis revealed that the soil contained 0.12 % total nitrogen, 1.41 % organic carbon and 27.65 mg kg-1 phosphorus. Nine months after oversowing the legumes, forages were harvested at 5 cm above ground level, chopped to < 1.5 cm, wilted for 4 hours and packed according to forage proportion. The proportion of the various forages harvested from oversown natural pastures prior ensiling process is shown in Table 1. This was then divided into four equal parts and ensiled in laboratory bottles (960 ml) silos with different additives (molasses, salt, molasses-salt and no additive) for 4 weeks at an ambient temperature of 26°C and tightly sealed.

Each silage treatment was replicated three times. After the period of ensiling, the silos were opened, examined and visually scored for the following physical properties: colour, odour, moisture, mould, pH and temperature according to the procedure of Bates (1998). Four independent scorers assessed and scored the silages. Samples were collected into sterile bottles for microbial identification and counts for bacteria, fungi and yeast according to method of Miles and Mistral described by Hedges (2002) and Cowan and Steel (1997). Samples were oven-dried at 65°C until constant weight. The oven-dried samples were then milled through a 1 mm sieve for analyses. Samples were analyzed for proximate composition and mineral contents according to the standard methods of AOAC (2006) while neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest *et al*. (1991). Hemicellulose was calculated as the difference between NDF and ADF.

The factors in the field comprised the following treatments, thereafter, different additives were added for silage making.

Table 1: Proportion of the various forages harvested from improved natural pastures prior ensiling process

TREATMENTS	GRASS (%)	LEGUMES (%)			
	Panicum maximum	*Stylosanthes giuianensis*	*Stylosanthes hamata*	*Desmodium intortum*	*Chaemocrista rotundifolia*
1	75.76	19.69	_	4.55	_
2	47.54	49.30	_	_	3.16
3	50.94	42.28	_	_	3.77
4	43.61	52.63	_	_	3.76
5	53.23	_	45.97	_	0.81
6	46.64	_	53.36	_	_
7	48.90	_	51.10	_	_
8	38.40	_	61.60	_	_

Treatment 1: Stylosanthes guianensis cv. Cook + Drilling + Zero tillage

Treatment 2: Stylosanthes guianensis cv. Cook + Drilling + Minimum tillage

Treatment 3: Stylosanthes guianensis cv. Cook + Broadcasting + Zero tillage

Treatment 4: Stylosanthes guianensis cv. Cook + Broadcasting + Minimum tillage

Treatment 5: Stylosanthes hamata cv. Verano + Drilling + Zero tillage

Treatment 6: Stylosanthes hamata cv. Verano + Drilling + Minimum tillage

Treatment 7: Stylosanthes hamata cv. Verano + Broadcasting + Zero tillage

Treatment 8: Stylosanthes hamata cv. Verano + Broadcasting +Minimum tillage

Statistical analysis:

The data collected were analysed using the General Linear Model procedure and the treatment means were separated using Duncan's Multiple Range Test via the SPSS Statistics 20 (IBM, 2011).

Results

The effects of legume species, tillage practice, sowing methods and additive types on the physical characteristics of the silage produced from oversown natural pastures are shown in Table 2. There were no differences (P>0.05) in the colour, odour, moisture, pH and temperature scores of the silages produced from the various treatments. The various additives used in this study influenced the level of mouldiness (P<0.05) and temperature (P<0.01) of the silage produced. Silage ensiled with molasses-salt as additive had a higher (P<0.05) value for mouldiness indicator, which indicated no presence of mould in the silage.

The interaction effect of species type and additive, species type and sowing method, species type and tillage, tillage method and sowing method, tillage operation and additive types, sowing method and additive showed significant (P<0.01) effects on the temperature of silage produced.

Table 3 shows the microbial population of silages produced from oversown pastures with or without additives. Silages produced from plots oversown with *S. hamata*, had higher (P<0.0001) total anaerobic and total lactic acid bacteria counts than those produced from plots oversown with *S. guianensis*. Silage produced using molasses as additive showed higher (P<0.05) total anaerobic counts than those produced with no additive. The broadcasting method of sowing legumes into natural pasture land yielded silage with higher (P<0.001) total lactic acid bacteria counts and total fungal counts than those with drilled legumes. Silage ensiled using molasses as additive showed the least total yeast count (P<0.05) than the rest of the treatments.

The interaction effect of species type and additive, species type and sowing method, species type and tillage, tillage method and sowing method, tillage operation and additive types, sowing method and additive showed significant (P<0.01) effect on the TAC, TLC, TFC and TYC content of silage produced.

The results of the proximate and fibre composition of the silages are shown in Table 4. Higher CP values (p<0.05) was recorded from silages made from pasture that was oversown with *S. hamata* legume (143.20 g/kg) than those oversown with *S. guianensis*. Silage produced from pasture land with minimum tillage showed higher CP content than those harvested from land with zero tillage. Silage that was made from forages that were broadcast with legume seeds recorded higher (P<0.001) CP contents than those that were drilled. Meanwhile, silage made from forage that were sown with drilling method produced higher (P<0.001) ADF content. Silage ensiled using salt and molasses-salt as additive showed higher (P<0.01) CP and EE contents than the rest treatments. Silage ensiled using molasses-salt as additive showed

Table 2: Effects of silage additives, sowing methods, plant varieties and tillage on the physical characteristics of silage made from oversown pasture

Factors	Colour	Odour	Moisture	Mould	pH	Temp
Species						
S. guianensis	7.52	26.83	9.33	7.51	4.02	26.56
S. hamata	7.63	26.65	9.33	8.04	4.02	26.50
S E M	0.13	0.14	0.08	0.05	0.17	0.055
Tillage						
Zero	7.56	24.64	9.34	8.09	4.07	26.58
Minimum	7.58	26.82	9.33	7.46	3.97	26.48
SEM	0.13	0.14	0.07	0.19	0.17	0.055
Sowing						
Broadcasting	7.52	26.88	9.35	7.86	4.06	26.47
Drilling	7.63	26.59	9.33	7.69	3.98	26.58
SEM	0.12	0.14	0.07	0.19	0.17	0.055
Additive						
Control	7.87	26.73	9.26	7.83^{ab}	4.54	26.20^{c}
Molasses	7.38	26.73	9.43	7.73^{ab}	3.53	26.46^{b}
Salt	7.63	26.73	9.26	7.31^{b}	4.51	26.69^{a}
Molasses-salt	7.42	26.76	9.39	8.23^{a}	3.49	26.76^{a}
SEM	0.26	0.20	0.11	0.2	0.024	0.077
P-value						
Species	0.5714	0.3886	0.8729	0.0389	0.3921	0.3224
Tillage	0.9098	0.3645	0.8729	0.0140	0.7910	0.3251
Sowing	0.5714	0.1612	0.8729	0.4866	0.5172	0.0932
Additive	0.2041	0.9994	0.5670	0.0927	0.1325	0.8793
Species x additive	0.0603	0.4533	0.6331	0.1661	0.3100	0.0000
Species x sowing	0.7382	0.4184	0.9943	0.0061	0.6600	0.0000
Species x tillage	0.9519	0.5107	0.9943	0.0101	0.9000	0.0000
Tillage x sowing	0.9519	0.3624	0.994	0.0006	0.8200	0.0000
Tillage x additive	0.5121	0.5960	0.6331	0.4857	0.2800	0.0100
Sowing x Additive	0.5121	0.5960	0.6331	0.4857	0.4500	0.0000
Species x tillage x sowing x additive	0.1274	0.5322	0.8007	0.0437	0.0600	0.5300

[a, b]: Means with the same superscript on the same column are not significantly different
SEM – standard error of means.

Table 3: Effects of silage additives, sowing methods, plant varieties and tillage on the microbial population (cfu/g) of silage made from oversown pasture

Factors	Total Anaerobic Count	Total Lactic Acid Count	Total Fungi Count	Total Yeast Count
Species				
S. guianensis	5.79[b]	5.92[b]	2.59[a]	5.39
S. hamata	6.04[a]	6.15[a]	0.64[b]	5.14
S E M	0.04	0.19	0.62	0.12
Tillage				
Zero	5.77[a]	6.04	1.62	5.47[a]
Minimum	6.055[b]	6.03	1.61	5.06[b]
SEM	0.28	0.05	0.33	0.11
Sowing				
Broadcasting	5.96	6.12[a]	0.91[a]	5.36
Drilling	5.86	5.96[b]	2.26[b]	5.17
SEM	0.12	0.28	0.33	0.11
Additive				
Control	5.67[b]	5.92	0.65[c]	5.46[a]
Molasses	6.05[a]	6.07	3.21[a]	4.74[b]
Salt	5.99[ab]	6.15	1.93[b]	5.47[a]
Molasses-salt	5.92[ab]	6.02	0.66[c]	5.39[a]
SEM	0.1	0.2	0.44	0.13
P-value				
Species	0.0007	0.0010	0.0001	0.2001
Tillage	0.0001	0.8851	0.9696	0.0314
Sowing	0.1464	0.0212	0.0011	0.2999
Additive	0.0014	0.1125	0.0001	0.0209
Species x additive	<.0001	0.0085	<.0001	0.0097
Species x sowing	0.0099	0.0008	<.0001	0.2350
Species x tillage	<.0001	<.0001	0.0008	0.0160
Tillage x sowing	0.0009	0.0642	<.0001	0.0162
Tillage x additive	0.0009	0.0240	0.0002	0.0150
Sowing x additive	0.0009	0.0240	0.0002	0.0150
Species x tillage x sowing x additive	<.0001	0.0052	<.0001	0.0008

[ab], means with the same superscript are not significantly different
SEM – standard error of means.

Table 4: Effects of silage additives, sowing methods, plant varieties and tillage on the proximate composition and fibre fractions (g/kgDM) of silage made from oversown pasture

Factors	DM	CP	EE	ASH	NDF	ADF	HEMI	P	Ca
Species									
S. guianensis	340.60	130.50^{b}	126.30	77.50	698.30	331.70	375.00	2.44^{b}	3.24^{b}
S. hamata	335.60	143.20^{a}	123.50	82.50	697.90	349.70	350.00	3.24^{a}	4.08^{a}
SEM	4.40	0.99	2.62	1.89	8.40	11.60	13.40	0.48	0.51
Tillage									
Zero	332.50	135.60^{b}	124.30	80.00	693.60	344.20	366.70	2.57^{b}	3.36^{b}
Minimum	343.70	138.10^{a}	125.40	80.00	710.80	335.40	358.30	3.11^{a}	3.97^{a}
SEM	10.70	1.35	2.73	2.02	18.0	9.10	6.00	0.17	0.19
Sowing									
Broadcasting	331.70	141.00^{a}	123.40	80.00	698.30	318.30^{b}	380.00	2.70	3.50
Drilling	344.50	132.70^{b}	126.00	80.00	706.30	361.30^{a}	345.00	2.98	3.82
SEM	4.60	1.23	2.71	2.02	8.80	11.40	13.90	0.17	0.19
Additives									
Control	334.50	134.10^{b}	114.60^{c}	80.00	710.00	341.70^{ab}	368.30	2.56^{b}	3.40^{c}
Molasses	326.0	136.10^{ab}	121.30^{bc}	79.20	693.30	309.20^{b}	384.20	2.29^{c}	3.04^{d}
Salt	343.10	138.90^{a}	129.60^{ab}	85.80	706.70	335.80^{ab}	370.80	3.13^{a}	3.94^{b}
Molasses-salt	348.10	138.90^{a}	134.20^{a}	75.00	699.20	372.50^{a}	326.70	3.38^{a}	4.26^{a}
SEM	6.10	1.92	3.56	2.32	11.70	15.80	19.60	0.23	0.25
P-value									
Species	0.4538	0.0001	0.4565	0.0743	0.4912	0.3053	0.2035	0.0003	0.0008
Tillage	0.0935	0.0259	0.7743	1.0000	0.1806	0.5802	0.6704	0.0127	0.0125
Sowing	0.0574	0.0001	0.5285	1.0000	0.5333	0.0078	0.0763	0.1893	0.1851
Additive	0.1145	0.0079	0.0012	0.0572	0.7888	0.0492	0.1892	0.0016	0.0025
Species x additive	0.0060	<.0001	<.0001	0.0813	0.9663	0.0011	0.0300	0.0001	0.0001
Species x sowing	0.0265	<.0001	0.2596	0.3815	0.2137	0.0459	0.1550	0.0001	0.0001
Species x tillage	0.3498	<.0001	0.2919	0.3815	0.4833	0.1348	0.2615	0.0003	0.0001
Tillage x sowing	0.0238	<.0001	0.5840	0.1372	0.3081	0.0718	0.2830	0.0001	0.0001
Tillage x additive	0.0207	0.4977	0.0026	0.3947	0.5812	0.2305	0.5342	0.0001	0.0001
Sowing x additive	0.0366	0.0007	0.0007	0.3947	0.0298	0.0035	0.0001	0.0001	0.0001

$^{a, b, c}$: values in the same column for each item with different superscripts differ significantly (P<0.05).
DM – Dry Matter, CP – Crude protein, EE – Ether extract, NDF - , ADF - , HEMI – Hemicellulose, Ca – Calcium, P - Phosphorus
SEM – standard error of means.

higher (P<0.01) ADF than those ensiled using molasses. The content of Ca ranged from 3.04 g/kg in silage with molasses additive to 4.08 g/kg in silages produced from plots oversown with *S. hamata*.

The interaction effect of species type and additive, species type and sowing method, species type and tillage, tillage method and sowing method, tillage operation and additive types, sowing method and additive showed significant (P<0.01) effect on the DM, CP, EE, ASH, NDF, ADF and hemicellulose

Discussion

The olive-green colouration of the silage in this study was in line with silage ranking by Bates (1998) and Babayemi (2009) and described as desirable and acceptable silage. The colour of the silage was close to the original colour of the grass, which was an indication of good quality silage that was well preserved (Oduguwa *et al*., 2007). Good silage usually preserves well the original colour of the pasture or any forage ('t Mannetje, 1999). Differences were not observed in the smell of the silage as all the silages were characterized by a pleasant smell as it fell between desirable and acceptable silage (Bates, 1998). Kung and Shaver (2002) reported that a pleasant smell is accepted for a good or well made silage. The moistness of silage is a good indicator of how well it is preserved as the moistness of the silages under study fell within the ranking of silage having no free water and slightly moist silage (Bates, 1998). This is also in line with the findings of Ukanwoko and Igwe (2012). The ranges of silages in this experiment that fell within slightly mouldy to mould-free silage according to Bates (1998) is a clear indication that the silages were well preserved. The silages made based on different *Stylosanthes* species, tillage and sowing methods with different additives were well preserved since they fell between fair silage and good silage as ranked by Bates (1998) which is a reflection of silage from tropical forages.

The pH value in the present study was within the range of 4.5 – 5.5 classified to be pH for good silage (Meneses *et al*., 2007). Generally pH is one of the simplest and quickest ways of evaluating silage quality. However, pH may be influenced by the moisture content and the buffering capacity of the original materials. Silage that has been properly fermented will have a much lower pH (be more acidic) than the original forage. Kung and Shaver (2002) in their interpretation of silage analysis stated that pH values of good quality grass and legume silage in the tropics ranges between 4.3 and 4.7.

Among all the additives used, silage with salt as additive had the highest count of lactic acid bacteria and this is in line with the findings of Neres *et al*. (2013) that reported higher count of lactic bacteria for Tifton 85 bermuda grass + salt above all other additives. The dominance of lactic acid bacteria in the silage made in this experiment was responsible for its good preservation. This agrees with the report of Nsereko *et al*. (2008) that the presence of lactic acid in silage improves animal performance

Fungi were identified in the silages made in this study which agreed with Neres *et al*. (2013) that fungi were present in Tifton 85 bermuda grass. However, fungi contents did not reach the minimum count of 30 cfu/g in silage in this study. This makes it safe for animal consumption (Neres *et al*., 2013). Fungi such as genera Penicillium and Aspergillus have been reported to be of public health concern because of production of mycotoxin and aflatoxin, which cause real hazards to animal health and are transferred to the milk of lactating cows (Neres *et al*., 2013). This has made them to be a major health concern in the use of both silage and hay for animal feeding. Fungi are aerobic and normally appear in large amounts in a period of aerobic deterioration, mostly after the growth of yeasts and aerobic bacteria (McDonald *et al*., 1991).

The results of the yeast counts in the silages made in this study fell within the same range reported by Neres *et al*. (2013) when Bermuda grass was ensiled with different additives. The anaerobic conditions and organic acids' concentration are two factors that affect yeast survival during silage storage

(Bravo-Martins *et al*., 2006), provided by the presence of oxygen in a silo (Jonsson and Pahlow, 1984). Yeasts are able to develop at low concentrations of oxygen (McDonald *et al*., 1991) and in a wide pH range (3 to 8) (Lima *et al*., 2002). According to Woolford (1990), yeasts are also able to ferment other sugars besides glucose. They have an extra source of energy in order to bear adverse effects of low pH and anaerobic conditions of a silo. Woolford (1990) considered that silages with yeast counts above 5.0 log cfu/g of silage are highly susceptible to deterioration.

The dry matter contents of all the silages fell between 250-350 g/kg DM which have been considered to be appropriate for good silage (Petterson, 1988). The CP content of silages with additives was on average higher than that of the control. The results obtained for CP contents for silages in this study were higher than those reported by Mtengeti *et al*. (2006) when studies on the effects of additives on silage quality were carried out. The results strengthen the possibilities of using locally available additives to improve and conserve nutritious fodder. From this study silage made from pastures that were oversown with *S. hamata* had higher CP content (143.20 g kg-1) than the one that was oversown with *S. guianensis*. However, the silage from forage harvested from plots with minimum soil tillage had 138.10 g kg-1 CP content which was higher than 135.60 g kg-1 recorded for zero tillage. The higher CP content of silage from forages harvested from minimally tilled plots could be due to increase in the rooting depths of the plants as a result of loosening of the soil structure which also improves soil aeration (Ojo *et al*., 2015). This could have enhanced the well-being of the rhizobia within the root zones of the legumes thereby increasing their nitrogen fixation and hence the quality of the forage (Dwyer *et al*., 1988). The CP recorded in this study surpassed the threshold of 60 g/kg required by rumen microbes to build their body protein (Van Soest, 1994). Silages made from plots oversown by broadcasting of seeds were found to have higher crude protein content as opposed to that planted by drilling. This could be as a result of plant spacing and environmental conditions while planting (Osuagwu and Edeoga, 2013). The observed differences in the proximate composition values in the legumes conform to the findings of Osuagwu and Edeoga (2103) that these differences might be due to environmental conditions and methods of planting. It also conforms to the findings of Khan *et al*. (2009) that varying concentration levels could partly be explained by differences between the forage species, levels of nutrients in the soil, influences of locality and climate, growth stage and season when forage sampling was done. Moderate fibre contents in this study, have been reported to facilitate the colonization of ingesta by rumen microorganisms which in turn might induce higher fermentation rates, enhance the intake and digestibility of tropical feeds by ruminants which will improve animal performance (Eastridge, 2006).

Tillage operation and addition of additives on the silage have resulted in an increase in the mineral contents of the silages produced in this study. Generally, silages produced from legumes have been reported to have higher Ca contents than those from grasses. This is in accordance to the report of Marschner (1993) that there is a marked difference between the level of Ca in legumes and grasses. The range of values recorded for Ca in the present study is higher than 0.7 – 0.9 g/kg DM reported earlier (Muhammad *et al*., 2005) but above the critical level of 3 g/kg DM recommended for ruminant needs (McDowell *et al*., 1993). The P level in this study is both above the critical level of 2.5 g/kg DM for ruminant animals and a mean value of 1.2 g/kg DM reported by Muhammad *et al*. (2005). Phosphorus contents of silage with molasses-salt additive was considerably higher than from other additive types. The higher mineral contents in silage made from forages harvested from minimum tillage area compared with zero tillage area might be due to pre-planting agricultural practices carried out on the farm land. It could also be due to increase in the rooting depths of the plants as a result of loosening of the soil structure which then improves soil aeration and hence

aids in tapping minerals from the soil for plants' benefits (Dwyer *et al*., 1988).

Conclusion

Silages made with molasses-salt additive are better in terms of odour. Silage made from pastures that are oversown with *S. hamata* had higher levels of lactic acid bacteria. The pH was within the range of values for good quality silage. Silage made from plots oversown with *S. hamata* had higher CP content. Ensiling forages with additives produced high quality silages. This study revealed that improvement of the natural pasture by oversowing with forage legumes will go a long way in enhancing the quality of the natural pasture for improved ruminants' production especially during the dry season.

Conflict of interest

The authors declare that we have no conflict of interest.

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Public brief

Improvement of natural pasture-land by carrying out necessary management practices such as tilling the soil and oversowing with proven forage legumes followed by conservation with nutritive improvement of silage, will provide high quality feed resources for ruminants especially during the dry season. This will ensure sustainable animal performance and productivity.

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PERFORMANCE AND ORGAN CHARACTERISTICS OF BROILER CHICKENS FED VARYING LEVELS OF RUMEN CONTENT

M Umar[1], Nuhu, Y.A[2], Yakubu Z M[2], Muazu M S[1] and Kirfi A M[1]
[1]Department of Animal Production, Abubakar Tafawa Balewa University, Bauchi.
[2]Pre-ND Department, Audu Bako College of Agriculture, Danbatta, Kano.

Abstract

A trial was carried out to evaluate the effect of replacing wheat offal with Rumen Content on the growth performance of broiler chickens. A total of one hundred and fifty (150) day old ZATECH broiler chicks were randomly allotted to five (5) dietary treatments containing 0%, 50% sundried, 100% sundried, 50% roasted and 100% roasted levels of inclusion of rumen content as a replacement to wheat offal. Each treatment was replicated three times with five (5) birds per replicate in a completely randomized design experiment. The trial lasted for eight (8) weeks. The results showed that there were no significant differences in the initial weights, final weights, daily feed intake, daily weight gain and feed conversion ratio at the starter phase and finisher phases. Furthermore, no significant differences in the initial weight, final weight, daily feed intake, and daily weight gain (1508-1346.60), (100.00-83.57) and (39.53-36.43) were observed in the overall performance respectively. However, there was a significant difference ($P<0.05$) in the feed conversion ratio with the highest being 2.69g and the lowest 2.33g in the overall performance. From these findings, it could be concluded that rumen content could replace wheat offal without any detrimental effect on the performance of broiler chickens.

Keywords: Rumen content, broiler, chicken and organ.

PERFORMANCES ET CARACTÉRISTIQUES ORGANIQUES DES POULETS DE CHAIR RECEVANT DES NIVEAUX VARIÉS DE CONTENU DE RUMEN

Résumé

Un essai a été réalisé dans le but d'évaluer l'effet du remplacement des abats de blé par du contenu de rumen sur les performances de croissance des poulets de chair. Au total, cent cinquante (150) poussins de poulets de chair ZATECH ont été répartis de manière aléatoire à cinq (5) traitements alimentaires contenant des niveaux d'inclusion de contenu de rumen à 0%, 50% séché au soleil, 100% séché au soleil, 50% rôti et 100% rôti en remplacement des abats de blé. Chaque traitement a été répété trois fois avec cinq (5) oiseaux par répétition dans une expérience conçue selon un schéma complètement randomisé. L'essai a duré huit (8) semaines. Les résultats ont montré qu'il n'y avait pas de différences significatives au niveau des poids initiaux, des poids finaux, de la consommation alimentaire quotidienne, du gain pondéral quotidien et de l'indice de consommation à la phase de démarrage et aux phases de finition. De plus, aucune différence significative au niveau du poids initial, du poids final, de la consommation alimentaire quotidienne et du gain pondéral quotidien (1508-1346,60), (100,00-83,57) et (39,53-36,43) n'a été observée dans la performance globale. Cependant, une différence significative ($P <0,05$) a été notée au niveau de l'indice de consommation, le plus élevé étant 2,69 g et le plus bas 2,33 g dans la performance globale. Sur la base de ces résultats, on pourrait conclure que le contenu du rumen pourrait remplacer les abats de blé sans aucun effet néfaste sur les performances des poulets de chair.

Mots-clés : contenu de rumen, poulet de chair, poulet et organe

Introduction

Background information

A major problem facing the development of broiler production is the availability and high cost of feedstuffs. A significant cost of production faced by poultry farmers is that of feed (55 – 70%) and because it is usually unaffordable by the poor peasant farmers, the output is generally poor, thus leading to a shortage in the availability of protein to the citizenry (Atteh, 2003). There is also competition between man and poultry for conventional feedstuffs like maize, wheat, soya bean among others. There is, therefore, the need for alternative and non-conventional feedstuffs to be used (Biobaku *et al*., 1999). Rumen content is a solid waste generated daily at abattoirs in Nigeria with about 50,000 metric tonnes available per year (Makinde, 2008). The content is made up of plant materials at various stages of digestion and is rich in microbial protein (Emmanuel 1978; McDonald *et al*., 1990).

The nutrient content and chemical composition of the diets destined for poultry feeding have been modified in the last decade with the aim of improving feed intake and productivity. An increase in nutrient concentration and digestibility of the ingredients together with a reduction in feed particle size to improve quality are some of the changes introduced. The implementation of these strategies has resulted in a decrease in crude fibre content of the diets and variations of the overall structure of the feed. The growth in Nigeria's poultry sector is constrained by the persistent scarcity and high cost of major feed inputs such as corn and soya bean meals. On the other hand, there is a need to increase animal productivity in order to make animal protein sources available and more affordable to Nigeria's populace. This could be enhanced by turning discarded rumen content to a useful source of fibre.

Problem Statement

The locally available fibre sources commonly used in poultry production are wheat and rice offal, maize bran and soya beans bran. However, these ingredients are sold at exorbitant prices leading to the high cost of poultry production in Nigeria. This makes it difficult for poultry farmers to continue production (Aremu *et al*., 2010).

However, in using fibre sources like rumen digesta, caution must be applied. The excessive use of fibre sources in the diets of poultry may increase the 'viscosity of the intestinal content with a resulting decrease in bioavailability of vitamin A' (Mendel, 2013) and utilization of dietary fat, which adversely affects body weight gain and carcass quality.

Justification

Poultry production in Nigeria has witnessed a series of developments particularly in the area of nutrition. Feed formulation involves the use of available feed ingredients to supply adequate amounts of nutrients required by different species of poultry. However, formulating feeds can only be possible when the available ingredients are not expensive. Rumen content from cattle and other ruminants like sheep and goats is a substantial waste that is readily available daily at abattoirs (Odunsi *et al*., 2004). Esonu *et al*. (2006) and Dairo *et al*. (2006) state that rumen content is the consumed plant material that ruminant animals ingest and is later harvested while it is at various stages of digestion. It is rich in protein and other micro-flora such as fungi, protozoa, and bacteria. Monogastric species cannot digest cellulose and other fibrous materials in rice milling waste, yet the available protein in rumen digesta can be utilized by broilers to obtain useful fat and other nutrients. Therefore the use of rumen digesta can reduce feed costs thereby increasing the rate of profit to the poultry producers.

Aims and Objectives of the Study

The study was conducted to determine the nutritional value of rumen content in the diets of broiler chickens with the following specific objectives:

1. To analyse the effects of replacing rumen content with wheat offal on the

performance of broiler chickens.
2. To determine the carcass and organ characteristics of boiler chickens when fed diets containing rumen content.

Materials and Methods

Experimental Site

The research was carried out at the poultry research farm of Bauchi State College of Agriculture. The college is located at Yelwan along Tafawa Balewa road in Bauchi Local Government Area of Bauchi State. The State lies between longitude 100101 to 301N and latitude 9041 and 100311E at an altitude of 6902 metres above sea level (BSADP, nd).

Experimental birds and their management

The poultry pen was swept, dusted and washed. All cobwebs were thoroughly cleaned and the pen was disinfected. The pen was partitioned and divided equally into 12 research pens. The rough cemented floor was covered with saw dust to a depth of 5cm. A total of 150 day old chicks were purchased from Zartech hatchery. All the birds were found healthy and in good condition. The chicks were brooded in two of the pens for two weeks and all the windows were covered to have the required heat for brooding. Cross ventilation was provided in and outside the pen. Heat for brooding was supplied with a lantern, which also served as a source of light when there was no electric light.

The birds were fed commercial feed during the two weeks of brooding. Feed and water were given *ad-libitum*. After the brooding period the chickens were randomly allotted to five experimental treatments, each treatment was replicated three times with ten birds per replicate. Vaccination of the birds with infectious bursal disease vaccine (IBDV) was conducted at the first week and repeated at third week, while Newcastle disease vaccine (Lasota) was administered at the second and fourth weeks. The experimental diet and water were given *ad-libitum* from the second week up to the eighth week of the study. Medications were also administered during this experimental period.

Experimental Diets

Five diets were formulated as diets A,B,C,D and E, each for the starter and finisher phases. Diet A for both the starter and finisher phases did not have rumen content (i.e control) while diet B,C,D and E contained rumen content as a replacement to wheat offal. Diet B and C comprised of sun dried rumen content at 5% and 10% levels of inclusion respectively, while roasted rumen content, also at 5% and 10% were in diets D and E respectively. The various diets were compounded manually; the starter contained 23% crude protein while the finisher contained 21% crude protein. The percentage composition and calculated analysis of the experimental diets are shown in the tables 1 and 2.

Experimental Design

The experimental design used was a completely randomized design (CRD). Five different diets formed the treatments and each treatment was replicated three times with ten birds per replicate.

Data Collection

The experiment lasted for eight (8) weeks during which data from the third to eighth week on several parameters were collected. Some of the data were collected on a daily basis while others were on a weekly basis. All feed given to the birds was measured and the leftover were also collected and subtracted from the feed offered in order to assess the feed intake by the birds. The feed weight was taken using a measuring scale in kilograms.

From the beginning of the experiment, the birds were randomly picked, weighed and the weights were recorded. The birds were weighed weekly thereafter and weight change was determined by subtracting the initial weight from the final weight.

Data Analysis

All data collected were subjected to analysis of variance using SPSS statistical package 21 and least significance differences (LSD) were differentiated as described by Steel and Torrie (1984).

Results

Performance of broiler chickens fed graded levels of rumen content based diets at the starter phase

The performance of broiler chickens fed graded levels of rumen content is presented in table 3. All values obtained were not significantly different among the treatments. The initial weight ranged from 118.75 g in birds on diet A to 123.75 g in chicks on diet B. The final weights obtained were 668.75 g, 666.67 g, 611.46 g and 639.58 g for diets A, B, C, D and E respectively. The daily feed intake varied from 49.42 g in chicks fed diet D to 50.74 g in birds on diet A. The daily weight gain was found to be 19.57 g, 18.53 g, 18.38 g, 19.24 g and 17.34 g for diet A, D, B, E and C respectively; however, all the daily weight gains were similar. The higher feed conversion value was obtained in birds on diets C while the lowest value was obtained in diet A.

Performance of Broiler Chickens fed Graded Levels of Rumen Content Based Diets at the Finisher Phase

Table 4 shows the performance of broiler chickens fed on graded levels of rumen content based diets at finisher phase. The initial weight ranged from 668.75 g in birds on diet A to 611.67 g in chickens on diet C. The means did not differ among the dietary treatments.

Similarly, values of the final weight were not statistically different with the final weights varying from 2350.4 g in birds on diet B to 2257.8 g in chickens on diet A. The feed intake, daily weight gain and feed conversion rations were also not different statistically.

The daily feed intakes were 151.59 g, 117.52 g, 137.59 g, 132.97 g and 135.45 g for diets A, B, C, D and E respectively. While the highest daily weight gain value was obtained in birds on diet B, the lowest value was obtained with diet C. Furthermore, the feed conversion ratio obtained in birds on diet C with a value of 2.50 was found to be higher compared to 1.96 on diet B.

Table 1: Composition of the experimental diet at starter phase 3-5 weeks

Ingredients	**Diets**				
		SRC		**RRC**	
	A (0%)	**B (5%)**	**C (10%)**	**D (5%)**	**E (10%)**
Maize	50.70	50.70	50.70	50.70	50.70
Soya bean meal	33.00	33.00	33.00	33.00	33.00
Wheat offal	10.00	5.00	-	5.00	-
Rumen content	-	5.00	10.00	5.00	10.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.50	1.50	1.50	1.50	1.50
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Toxail binder	0.10	0.10	0.10	0.10	0.10
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

SRC= sun dried rumen content; RRC= roasted rumen content

Table 2: Composition of the experimental diet at finisher level (5 – 8 weeks)

Ingredients	Diets				
	SRC			RRC	
	A (0%)	B (5%)	C (10%)	D (5%)	E (10%)
Maize	49.60	49.60	49.60	49.60	49.60
Soya bean meal	29.10	29.10	29.10	29.10	29.10
Wheat offal	15.00	7.50	-	7.50	-
Rumen content	-	7.50	15.00	7.50	15.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Toxil binder	0.10	0.10	0.10	0.10	0.10
Bone meal	2.00	2.00	2.00	2.00	2.00
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

SRC= sun dried rumen content; RRC= roasted rumen content

Table 3: Performance of Broiler Chickens fed Graded Levels of Rumen Content Based Diet at the Starter Phase.

Parameter	SRC			RRC			
	A (0%)	B (5%)	C (10%)	D (5%)	E (10%)	SEM	LS
Initial weight	118.75	123.75	116.88	120.63	115.92	1.20	NS
Final weight	668.75	666.46	611.46	49.42	600.46	56.00	NS
Daily feed intake	50.74	49.62	49.59	49.42	49.45	1.34	NS
Daily weight gain	19.57	18.38	17.34	18.53	18.35	1.30	NS
FCR	2.26	2.87	2.87	2.66	2.75	0.35	NS

SEM= Standard Error of Mean; NS= Not Significant; FCR= Feed Conversion Ratio, SRC= sundried rumen content, RRC= roasted rumen content

Table 3: Performance of Broiler Chickens fed Graded Levels of Rumen Content Based Diet at the Starter Phase.

Parameter	SRC			RRC			
	A (0%)	B (5%)	C (10%)	D (5%)	E (10%)	SEM	LS
Initial weight	688.75	666.67	611.46	639.58	661.54	36.56	NS
Final weight	2257.80	2350.40	2081.70	224.70	221.55	76.10	NS
Daily feed intake	151.59	117.52	137.59	132.97	135.69	13.50	NS
Daily weight gain	58.26	60.71	55.35	57.92	56.99	3.50	NS
FCR	2.47	1.96	2.50	2.45	2.15	0.23	NS
Mortality	6	9	4	8	5		

SEM= Standard Error of Mean; NS= Not Significant; FCR= Feed Conversion Ratio, SRC= sundried rumen content, RRC= roasted rumen content

Overall Performance of Broiler Chickens fed Graded Levels of Rumen Content Based Diets

The performance of broiler chickens fed graded levels of rumen content is presented in table 5. All values were not significantly different between the dietary treatments. The final weight of the birds ranged from 1508.50 g on diet B to 1346.60 g for birds on diet C. The daily feed intakes were 100.00 g, 83.57 g, 93.56 g, 91.29 g and 92.55 g for diets A, B, C, D and E respectively.

The daily weight gain varied from 39.53 g in birds fed diet B to 36.34 g for birds on diet C. The highest feed conversion values were obtained in birds on diet C with a value of 2.69 and the lowest in diet B with a value of 2.33 g.

Table 5: Overall Performance of Broiler Chicken fed Graded Levels of Rumen Content Based Diets

Parameter	SRC		RRC				
	A (0%)	B (5%)	C (10%)	D (5%)	E (10%)	SEM	LS
Final weight	1485.5	1508.5	1346.6	1443	1453.11	39.36	NS
Daily feed intake	100.00	83.37	93.56	91.29	92.21	4.43	NS
Daily weight gain	38.92	39.34	36.34	38.24	37.59	1.50	NS
FCR	2.37	2.33	2.69	2.48	2.55	0.08	*
Mortality	0	0	0	0	0	0	

SEM= Standard Error of Mean; NS= Not Significant; FCR= Feed Conversion Ratio, SRC= sundried rumen content, RRC= roasted rumen content

Based on the study findings, the data on the carcass weights of broilers at the end of the experiment (Table 6 below) revealed that there was no significant difference in the live weights of the broilers between the different treatments. However, birds fed with diet E had the least live weight (2100 g) whereas the highest value was recorded in birds fed with the control diet. A weight of 2350 g was obtained for both diets B and D. The plucked weights indicated that there was a significant difference ($p<0.05$) between the treatments. The weights of birds fed with diet C were similar to those of birds fed with diet D.

The highest plucked weights were recorded in birds fed with the control diet (2220 g) followed by birds fed with diet B (1999 g) while the least were observed in birds fed with diet E (1704 g). The carcass weights of the broilers revealed that there was no significant difference ($p>0.05$) between all the treatments. Birds in the control diet with a 0% inclusion level of rumen content had the highest carcass weight (1602 g) while the least was recorded in 10% SRC (diet C) with 1258 g.

The organ weights, expressed as a percentage of live weights are shown in Table 6 above. there was a significant difference ($p<0.05$) on values obtained for liver weights between the different treatments, with the highest percentage being recorded among birds fed the control diet while the least value was recorded in diet C (1.43%) and then diet E with 1.48%. There were no significant differences ($p>0.05$) in the heart weights between the treatments, with the highest percentage (0.82%) value recorded in birds fed diets without the rumen content (control) whereas the least (0.43 %) was recorded at both diets of the RRC.

On the other hand, the spleen weight values were significantly different ($p<0.05$) between the treatments, with the control diet having the highest spleen weight (0.93%) followed by the 5% SRC (diet B) with 0.74%. The least value (0.55%) of spleen weight was obtained with diet E.

The gizzard weights in relation to live weights indicated a non-significant difference between the treatments ($p>0.05$). Birds fed with 5% sun dried rumen content (diet B) had the highest value of 4.3% of gizzard weight followed by the control group while the least among the values obtained was observed in birds fed 5% RRC (3.210%). The 10% SRC and

Table 6: Carcass, organs and gut weight expressed as percentage live weight

Parameters	SRC			RRC		
	A (0%)	B (5%)	C (10%)	D (5%)	F (10%)	SEM
Final live weight (g)	2450	2350	2200	2350	2100	0.229^{NS}
Pluck weight (g)	2220^{c}	1999^{b}	1941^{ab}	1803^{ab}	1704^{a}	0.209*
Carcass weight (g)	1602	1424	1258	1277	1302	0.272^{NS}
Dressing %	65.44	60.81	57.77	54.34	62.13	0.721^{NS}
Liver weight (%)	2.54^{c}	1.57^{ab}	1.43^{a}	1.64^{a}	1.48^{ab}	0.00*
Heart weight (%)	0.82^{b}	0.75^{b}	0.50^{a}	0.43^{a}	0.43^{a}	0.001*
Spleen weight (%)	0.93^{c}	0.75^{b}	0.57^{ab}	0.58^{ab}	0.55^{a}	0.010*
Gizzard weight (%)	4.10^{b}	4.22^{b}	3.86^{ab}	3.21^{a}	3.90^{ab}	0.093^{NS}
Large intestine weight (%)	5.52^{b}	4.42^{a}	3.78^{a}	3.70^{a}	3.82^{a}	0.009*
Small intestine weight (%)	1.47	1.49	1.34	1.28	1.33	0.793^{NS}
Head and leg weight (%)	6.79	5.58	5.21	5.11	5.45	0.284^{NS}

^{a,b,c}=Means in the same row with different superscripts are significantly different (p>0.05;) NS = Not Significant, SEM = Standard Error of Mean; SRC= Sundried Rumen Content; RRC= Roasted Rumen Content

10% RRC (C and E) diets recorded values of 3.86% and 3.90% respectively.

The results also indicated that there were significant differences (p<0.05) in the large intestine weights recorded. Birds with 5.52% on the control treatment had the highest value while birds fed with 5% RRC had the least percentage large intestine weights of 3.70%.

The small intestine weights had no significant differences between the treatments and the weights ranged from 1.49% to 1.28% of live weight, where the highest value was recorded for birds fed with the 5% SRC diet. Furthermore, birds fed with 10% SRC had the least percentage of the head and leg weights (5.21%) whereas the highest percentage of head and leg weights were recorded for birds fed with the control diet (6.790%). There were no significant differences (p>0.05) in the percentage weights of legs and heads of the broilers on different treatments.

Discussion

The daily feed intake was not significantly different between all the dietary treatments in the starter (49.42-50.74 g) and finisher (117.52-151.59 g) phases. This result was in conformity with the findings of Adeniji and Jimoh (2007), who fed different inclusion levels of rumen content to pullet chicks and obtained similar values of feed intake.

The daily weight gains observed at the starter (49.42-50.74 g) and finisher (117.52-151.59 g) phases were not affected by the dietary levels of inclusion of rumen content. A similar trend was also found at the overall weight gain (36.34-39.53 g). In contrast to these findings, Yakubu *et al* (2007) reported that, broiler chickens fed urea treated rumen content at 12% showed a significantly higher carcass yield and liver weight. This difference could be due to the urea treatment done to the rumen content.

The feed conversion ratio at the starter (2.26-2.87) and finisher (1.96-2.5) phases were statistically similar among the dietary treatments. However there was a significant difference among the treatments at the overall feed conversion ratio.

The study values obtained for the final live weight indicated that there were no significant difference (p>0.05) in the final live weight of broilers fed with differently processed rumen content. The findings agreed with the earlier work of Gwayo *et al*. (2006) who reported no significant difference in the final live weight of broilers fed different inclusion levels of goat rumen digesta. Also,

the finding is in line with the research made by Elfaki *et al*. (2015) who reported that there is no significant difference on the live weight of broilers fed with dietary processed dried rumen content.

The findings indicate that the significant difference ($p<0.05$) among the treatments on the percentage plucked weight does not agree with the observation made by Gwayo *et al*. (2006), who observed no significant difference between the control group and the treatment groups at both starter and finisher phases when birds were fed with rumen digesta as a replacement for wheat offal as a dietary fibre source.

The experiment carried out on the carcass weights had no significant ($p>0.05$) effect on the percentage live weights of broilers fed with sun dried and roasted rumen content. This agreed with the earlier work of Elfaki *et al*. (2015) who observed no significant effect on the carcass weights of broilers fed dietary treated rumen content.

Table 6 shows the organ weights of broilers at the end of the research. The gizzard weight was not different between all means and ranged from (4.22 to 3.21%). On the other hand, Gwayo *et al*. (2006) reported differences in weights of gizzard and the heart while all other organs were not significantly different. This may likely be due to differences in the processing method and the source of biodigesta between the two studies. However the results agreed with the findings made by Elfaki *et al*, (2015) who reported no significance difference in the weights of spleens between the treatment groups. The result on the weights of the small intestines indicated no significant difference ($p > 0.05$) between the treatment means and thus agrees with observations made by Elfaki *et al*, (2015) who also reported a non-significant effect on weights of the small intestine of birds in their study.

Conclusion

The results of the study indicated that dried rumen content can be incorporated in broiler diets at 10% replacement level of wheat offal without adverse effects on carcass yield. Therefore, using rumen bio-digesta in poultry diet could reduce the cost of feeding and subsequently prevent environmental pollution which the bio-digesta may have caused. Furthermore, up to 10% of dried rumen content can be used as a cheap source of energy and protein for poultry, though, its inclusion must be done with caution, as studies have not yet determine the microbial impact it might have. As such, the need for more researches cannot be over emphasised.

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GENOTYPIC AND SEASONAL VARIABILITY ON THE REPRODUCTIVE PERFORMANCE OF TWO STRAINS OF HYBRID LAYERS IN SOUTHWEST NIGERIA

Y I Irivboje[1,2], A O Fafiolu[1,2], M T Sanni[2], O A Irivboje[1,2] and C O N Ikeobi[1,2]
[1]World Bank Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, PMB 2240, Nigeria
[2]College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, PMB 2240, Nigeria

Abstract

The egg-laying or layer strain is of high nutrient and of good economic importance to the society at large due mainly to its egg production traits and also for its meat. The exotic layer strains have been able to adapt to the climatic and environmental conditions of the southern part of Nigeria. Non-the-less, challenges are still being faced in its rearing and production especially during extreme climatic conditions. Previous research found that changes in the seasonal environment had significant effects on egg fertility, hatchability of total set eggs and hatchability of fertile eggs. This study aimed at determining the effects of genotype and season on two exotic layer chicken strains. A total of one thousand five hundred (1500) layers per strain were used for the evaluation of their reproductive performances. Each strain included one hundred and twenty cocks (120) for random mating. It was observed that there were significant differences ($P<0.05$) in the values obtained for the different seasons. The late wet season had a higher significant difference ($P<0.05$) in values of the percent fertile (78.12±0.51), percent hatched (72.36±0.74) and percent hatchability (92.92±0.36) than other seasons. the percent hatchability (90.60±0.48), total hatched (67.68±0.98), percent fertility (74.80±0.72), were highly significant ($P<0.05$) in Brown dominant than the Hyline brown strain of laying Chicken with percent hatchability (88.38±0.49), total hatched (63.01±0.92) and percent fertility (71.02±0.71). In conclusion, it was discovered that the Brown dominant layer chicken strain had a better performance in the fertility and hatchability than the Hyline brown chicken layer and the late wet season was observed to be more favourable to percentages hatched, fertility and hatchability. The Brown dominant strain is preferable for brown layer production and also, the late wet season should therefore be targeted for optimal production of layers in southwest Nigeria.

Keywords: Reproductive, performance, Brown, dominant, Hyline, Hatchability, Fertility, Strain, Season

VARIABILITÉ GÉNOTYPIQUE ET SAISONNIÈRE SUR LA PERFORMANCE REPRODUCTIVE DE DEUX SOUCHES DE PONDEUSES HYBRIDES DANS LE SUD-OUEST DU NIGERIA

Résumé

La souche de poules pondeuses est très nutritive et revêt une importance économique pour la société dans son ensemble, principalement en raison de ses caractéristiques de production d'œufs et de sa viande. Les souches de pondeuses exotiques ont pu s'adapter aux conditions climatiques et environnementales de la partie sud du Nigéria. Néanmoins, des défis restent à relever dans leur élevage et production, en particulier dans des conditions climatiques extrêmes. Des recherches antérieures ont révélé que les changements de l'environnement saisonnier avaient des effets importants sur la fertilité des œufs, la capacité d'éclosion des œufs pondus au total et le taux d'éclosion des œufs fertiles. Cette étude visait à déterminer les effets du génotype et de la saison sur deux souches de poulets exotiques. Au total, mille cinq cent (1500) pondeuses par souche ont été utilisées pour l'évaluation de leurs performances reproductives. Chaque souche comprenait cent vingt coqs (120) pour un accouplement aléatoire. Des

Corresponding author email: youngiriv@yahoo.com

différences significatives (P <0,05) ont été notées dans les valeurs obtenues pour les différentes saisons. La saison humide tardive a eu une différence significative plus élevée (P <0,05) dans les valeurs du pourcentage de fertilité (78,12 ± 0,51), du pourcentage d'œufs éclos (72,36 ± 0,74) et du pourcentage de capacité d'éclosion (92,92 ± 0,36) par rapport aux autres saisons. Le pourcentage d'éclosion (90,60 ± 0,48), le total d'œufs éclos (67,68 ± 0,98), le pourcentage de fertilité (74,80 ± 0,72) étaient significativement (P <0,05) élevés chez la souche Brown dominante par rapport à la souche brune Hyline de pondeuses - pourcentage d'éclosion (88,38 ± 0,49), total éclos (63,01 ± 0,92), pourcentage de fertilité (71,02 ± 0,71). En conclusion, il a été découvert que la souche de pondeuse dominante brune avait une meilleure performance en matière de fertilité et d'éclosion par rapport à la pondeuse brune Hyline, et la saison humide tardive s'est avérée plus favorable aux pourcentages d'œufs éclos, de fertilité et d'éclosion. La souche dominante Brown est préférable pour la production de pondeuses brunes, en outre, la saison humide tardive devrait donc être ciblée pour une production optimale de pondeuses dans le sud-ouest du Nigeria.

Mots-clés : Reproductif, performance, Brown, dominant, Hyline, éclosion, fertilité, souche, saison

Introduction

The egg-laying or layer strain is of high nutrient and economic importance to the society at large due mainly to its egg production traits and also for its meat. The commercial layer is best known for table egg production because of the high level of genetic improvement in its laying performance and thorough management input (Ogbu, 2012). Hyline brown parent stock is expected to attain the weight of 1450 – 1530g with a feed intake of 81 – 85 g/day per bird at 18 weeks of age (Hyline, 2014). The Brown dominant strain is colour-sexed through silver-red S/s alleles of Silver gene. Brown dominant pullet at 18 weeks of age, with an average feed consumption of 79 g/day, is able to attain a body weight of 1450 to 1500g provided good management procedures and practices are adhered to (SochĹŻrek, 2008). At laying period, its livability is 95 – 97%.

Climate change is a natural process that takes place simultaneously on various time scales, in relation to the variation over time of the global climate or local climates, which may be the results of both natural forces and human activities (FAO, 2019). The exotic layer strains have been able to adapt to the climatic and environmental conditions of the southern part of Nigeria, non-the-less, challenges are still faced in its rearing and production especially during extreme climatic conditions. These challenges include; the effects of heat stress that has resulted in increased mortality of the birds, susceptibility to infections and diseases, drop in daily egg production, decrease in hatchability and fertility among others.

Heat stress has negative effects on both hatchability and fertility in poultry production. Previous research demonstrated that high environmental temperatures commonly called heat stress adversely affected egg production, fertility (McDaniel *et al*., 1995; Obidi *et al*., 2008) and hatchability (Lourens *et al*., 2005) of breeders. This was in line with other research work that showed that changes in the seasonal environment had significant effects on egg fertility (Aggarwal, 1987; Pruthi and Aggarwal, 1987; Das and Ali, 1999), hatchability of total set eggs (Farooq *et al*., 2003; Chowdhury *et al*., 2004), and hatchability of fertile eggs (Kalita *et al*., 1985; Sreenivasaiah and Joshi, 1987) in poultry and ducks.

Nigeria, like the rest of West Africa and other tropical lands, has only two seasons. These are the dry and the rainy seasons (Oguntunji *et al*., 2008). The Nigeria season has also been further divided into four by many researchers as; January – March being Late Dry season, April – June being Early Wet season, July – September being Late Wet season, October – December being Early Dry season (Adedeji *et al*., 2006). The seasonal variability is prevalent in the entire landscape of Nigeria including the southwest region. The seasons are therefore targeted by poultry farmers during their production cycle to maximise performance.

The objective of this study was to determine the effects of genotype and season on two exotic layer chicken strains; Brown

dominant and Hyline brown, and to compare their performances in the different seasons of the year.

Materials and Methods

Experimental Site

The study was carried out in a poultry breeding farm, located in Igboora, Oyo State, South-Western, Nigeria. Igboora is a town situated 80 km North of Lagos State with coordinates 7°26'10" N and 3°17'34" E. The vegetation of the area is typical of a Sahel savannah with two main seasons consisting the rainy and dry seasons.

Experimental Birds

Two strains of hybrid layers were used for this study; Brown dominant and Hyline brown. The birds were housed separately per genotype in a deep litter system of the production unit of the farm. Small wooden cages were provided in the pen for egg collection.

Egg collection, incubation and management

A total of one thousand five hundred (1500) layers per strain were used for the evaluation of their reproductive performances. Each strain included onehundred and twenty (120) cocks for random mating. Egg collection started when the layers were thirty (30) weeks old. Four hundred (400) eggs per strain (Brown dominant and Hyline brown) were collected for incubation per week (nine weeks per season) throughout the duration of the study. The four seasons under consideration were early wet, late wet, early dry and late dry. A total of three thousand six hundred (3600) hatchable eggs per strain were collected per season from the Breeder farm in Oyo state. The eggs were grouped to differentiate between batches and stored in the cold room at a temperature of 17°C prior to setting in the incubator. Before setting in the incubator, the eggs were sorted, arranged into trays and then aligned into trolleys. The eggs were positioned in the trays with the broad ends up to allow for ease of gas exchange (CO2 and O2) between the eggs and the environment. The trolleys were then moved to the fumigation chamber where the eggs were fumigated using formaldehyde (40%) and potassium permanganate crystals at a ratio of 2:1.

The hatchery unit is automated with a two stage incubation system, comprising the setters and the hatchers. After 18 days of incubation in the setter, the eggs spent a further 3 days in the hatcher. The temperature in the setters was set at 99.5°F and the relative humidity at 83.0% while the hatchers were set to a temperature of 98.5°F and 85.0% relative humidity. The ambient temperature was kept cool with air-conditioners installed in the incubator rooms. The setters allowed for the turning of the eggs at 60° hourly, sprinkling of humidified water, provision of heat to keep the air warm, the inflow of chilled water from the chiller to regulate the temperatur, and a damper to allow for the exchange of air between the inside of the incubator and the environment.

Candling of the eggs was carried out to determine the percentage fertility of the eggs on the 7th and 18th days of incubation. During the process, the eggs were separated into three groups; Fertile, Infertile and Dead-in-germ eggs, while records were taken on weekly basis.

After the candling operation, the fertile eggs were transferred into the hatchers in preparation for hatching. After hatching the chicks were grouped into three during counting and boxing and documented as follows: the normal chicks also termed real chicks, the reject chicks (abnormal chicks) and the dead-in-shell. Chicks which were under sized, poorly feathered, parrot beaked, blind, lame, and those with poorly absorbed yoke were considered and counted as rejects.

Estimation of percentage fertility, hatchability of fertile eggs, hatchability of set eggs and dead in shell

The percentage fertility, infertility, hatched, dead-in-Shell and hatchability were estimated using the formulae below:

$$\text{Fertility (\%)} = \frac{\text{Number of fertile eggs}}{\text{Total number of eggs set}} \times 100\%$$

$$\text{Infertility (\%)} = \frac{\text{Number of infertile eggs}}{\text{Total number of eggs set}} \times 100\%$$

$$\text{Hatchability (\%)} = \frac{\text{Number of eggs hatched out}}{\text{Total number of fertile eggs}} \times 100\%$$

$$\text{Hatched (\%)} = \frac{\text{Number of eggs hatched out}}{\text{Total number of eggs set}} \times 100\%$$

$$\text{Dead-in-Shell (\%)} = \frac{\text{Number of Dead-in-Shell}}{\text{Total number of fertile eggs}} \times 100\%$$

Statistical analysis

Data obtained were analysed using the General Linear Model of SAS (2009). After the removal of non-significant interactions, the following model was used:

$$Y_{ij} = \mu + S_i + T_j + \varepsilon_{ij}$$

Where, Y_{ij} = an observation of the trait (%Fertility, %Hatchability etc.),
μ = Overall mean
S_i = Effect of Strain (Brown dominant, Hyline brown)
T_j = Effect of Season (Early wet, Late wet, Early dry, Late dry)
ε_{ij} = Random error

The significant differences among treatments were determined by Least Significant Difference (LSD) test.

Statement on the welfare of the animals

Ethical approval: The experiment was conducted following the code of ethics for animal experimentation with prior approval by the University's Animal Ethics Committee.

Results

The effect of genotype on the reproductive performance of both layer birds

The effect of strain on the reproductive performance of both Brown dominant and Hyline brown is presented in Table 1. The result shows that the percent hatchability (90.60±0.48), total hatched (67.68±0.98), percent fertility (74.80±0.72), were significantly higher (P<0.05) in Brown dominant compared to Hyline brown strain of laying Chicken. However, the percent infertile (28.99±0.71) and percent dead-in-shell (11.62±0.49) were significantly higher (P<0.05) in Hyline brown than in Brown dominant.

Table 1: Effect of genotype on reproductive performance of both Brown dominant and Hyline brown strains of laying Chicken

Parameters	Brown dominant	Hyline brown
%Infertile	25.20±0.73[b]	28.99±0.71[a]
%Fertile	74.80±0.72[a]	71.02±0.71[b]
%Hatched	67.68±0.98[a]	63.01±0.92[b]
%D.I.S.	9.40±0.48[b]	11.62±0.49[a]
%Hatchability	90.60±0.48[a]	88.38±0.49[b]

[a, b]: means on the same row having different superscripts are significantly (p<0.05) different, D.I.S. – Dead in shell

Table 2: Effect of season on reproductive performance of both Brown dominant and Hyline brown strains of laying Chicken

Parameters	Late Dry	Early Wet	Late Wet	Early Dry
%Infertile	31.68±0.58[a]	27.43±0.92[b]	21.88±0.51[c]	27.39±1.01[b]
%Fertile	68.33±0.58[c]	72.58±0.91[b]	78.12±0.51[a]	72.62±1.01[b]
%Hatched	59.37±0.75[c]	65.55±1.10[b]	72.36±0.74[a]	64.12±1.32[b]
%D.I.S.	12.96±0.59[a]	10.09±0.56[c]	7.09±0.36[c]	11.91±0.61[a]
%Hatchability	87.04±0.59[c]	89.91±0.56[b]	92.92±0.36[a]	88.09±0.61[c]

[a, b, c]: means on the same row having different superscripts are significantly (p<0.05) different, D.I.S. – Dead in shell

The effect of season on the reproductive performance of both layer birds

The effect of season on the reproductive performance of both Brown dominant and Hyline brown is presented in Table 2. It was observed that there were significant differences ($P<0.05$) in seasonal variability. The late wet season was significantly higher ($P<0.05$) in values for the percent fertile (78.12±0.51), percent hatched (72.36±0.74) and percent hatchability (92.92±0.36) than other seasons. This was closely followed by the early wet season.

Discussion

The effect of genotype was highly significant as found in this study. The Brown dominant strain had a better reproductive performance in the percentage hatchability, fertility and hatched when compared to the Hyline brown which on the other hand had a significantly higher percentage in the total infertile, rejected chicks and dead-in-shell. This is in line with the works of Sola-Ojo and Ayorinde (2011) and Ndofor-Foleng (2015) whose results recorded significant effect of genotype on fertility and hatchability. The significance effect of genotype recorded in this study could have also be as a result of the acclimatization of the Brown dominant to the Nigerian environment since they have been used for production in the research farm for a longer period than the Hyline brown which were recently introduced into Nigeria from the United Kingdom to supplement the production of brown chicks in the breeder farm. It has been reported by Dauda *et al*. (2006) that the Nigerian climatic environment is characterised by high temperature and relative humidity typical of tropical regions which could negatively affect the physiological functions of birds.

The effect of season on reproductive performances of Brown dominant and Hyline brown was significant in the percent infertile, percent fertile, percent dead-in-shell, percent hatched and percent hatchability. The highest significant differences found in the percent fertile, percent hatched and percent hatchability productions were in the late wet season. This was closely followed by the early wet season. The percent infertile and percent dead-in-shell were highest in the early dry and late dry seasons. This could be as a result of the influence of season on fertility and hatchability (Olawumi, 2007) which made the lower temperature and favourable condition of the weather experienced during the wet season to give advantage to the reproductive performances of the layers while the harsh and hot environmental condition as a result of the dry season had a negative influence on the layers. The lower hatchability percentage recorded in the early and late dry seasons could be as a result of development of the embryo prior to incubation due to high environmental temperature which also weakens it. Jesuyon and Oseni (2015) reported that the best fertility and hatchability results were obtained in Black Nera and Isa Brown genotypes during the late wet season respectively. Also, earlier reports had also lay claim on the fact that reproductive performance of poultry was influenced by season. This is supported by Elsayed (2009) who reported that fertility in Ostrich was influenced by the season of production. Roy *et al*. (2003) also reported that season had significant effect on the fertility and hatchability of White Leghorn eggs. Similarly, Gonźalez-Redondo (2006) reported that laying date had influence on the fertility and hatchability of red-legged Partridge (Alectoris rufa) eggs. This influence of season was also similar to the results obtained in the study for Brown dominant and Hyline brown layers.

The results revealed that genotype had significant effect on the reproductive performance as the Brown dominant chicken layer strain had a better performance in the fertility and hatchability than the Hyline brown chicken layer. It was also found from the study that season had significant effect on the reproductive performance of both strains of laying birds. The late wet season was observed to be more favourable to percentage hatched, percentage fertility and percentage hatchability. On the other hand, the late dry season had

more impact on the percentage infertile and percentage dead-in-shell. It is therefore recommended that the Brown dominant strain is preferred for brown layer egg production in tropical condition and also that the late wet season should be targeted for optimal production of layers in southwest Nigeria.

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Conflict of interest statement

There is no conflict of interest with any individual or organization regarding the materials discussed in the manuscript.

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PERFORMANCE OF WEANED FEMALE RABBITS FED DIET CONTAINING VARYING LEVELS OF NEEM LEAF MEAL

*Egbeyale L T[1], Oni A O[2], Ayo-Ajasa O Y[1], Abiona J A[3], Adeleye O O[1], Oyebo A T[2] and Oyedare K S[4].
[1]Department of Animal Production and Health, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria.
[2]Department of Animal Nutrition, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria.
[3]Department of Animal Physiology, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria.
[4]Federal College of Animal Health and Production Technology, Moore Plantation, Ibadan, Oyo State.

Abstract

A thirteen weeks' experiment was conducted to determine the growth performance, blood profiles and carcass characteristics of female growing rabbits fed concentrate diets, containing neem leaf (*Azadirachta indica*) as replacement to wheat offal. A total of thirty six mixed breed weaned female rabbits were used for the study. The animals were divided based on weight equalization to T1 (control), T2 (5% NLM), T3 (10% NLM) and T4 (15% NLM). They were fed for thirteen (13) weeks with; feed and water given *ad libitum*. Data on growth, haematological, serum biochemical and carcass indices were collected and analysed using analysis of variance (ANOVA) and means separated using Duncan Multiple Range Test. The results showed that final weights and weight gain decreased ($p<0.05$) at 15% inclusion level of neem leaf meal (NLM). The haematological and serum biochemical parameters revealed no significant ($P>0.05$) differences for all the parameters measured. Only the head was significantly ($p<0.05$) influenced among all the carcass traits. It was concluded that replacement of wheat offal with neem leaf meal in the diet of female growing rabbits should not exceed 10% for optimum performance without causing any health challenge to the animals.

key words: neem leaf meal, weaned rabbit blood profile

PERFORMANCE DE LAPINES SEVRÉES NOURRIES AUX ALIMENTS CONTENANT DES NIVEAUX VARIABLES DE FARINE DE FEUILLES DE NEEM

Résumé

Une expérience de treize semaines a été menée pour déterminer les performances de croissance, les profils sanguins et les caractéristiques de carcasse des lapines en croissance recevant des régimes concentrés, contenant des feuilles de neem (Azadirachta indica) en remplacement des abats de blé. Au total, trente-six lapines sevrées de race mixte ont été utilisées pour l'étude. Les animaux ont été divisés sur la base de l'égalisation du poids en : T1 (témoin), T2 (5% NLM), T3 (10% NLM) et T4 (15% NLM). Elles ont reçu pendant treize (13) semaines des aliments, l'eau étant donnée ad libitum. Les données sur les indices de croissance, hématologiques, biochimiques sériques et de carcasses ont été collectées et analysées en utilisant l'analyse de variance (ANOVA) et les moyennes séparées à l'aide du Duncan Multiple Range Test. Les résultats ont montré que les poids finaux et le gain pondéral diminuaient ($p <0,05$) à un niveau d'inclusion de 15% de farine de feuilles de neem (NLM). Les paramètres hématologiques et biochimiques sériques n'ont révélé aucune différence significative ($P> 0,05$) pour tous les paramètres mesurés. Seule la tête a été significativement ($p <0,05$) influencée parmi tous les caractéristiques de la carcasse. Il a été conclu que le remplacement des abats de blé par de la farine de feuilles de neem dans l'alimentation des lapines en croissance ne devrait pas dépasser 10% afin d'obtenir des performances optimales sans nuire à la santé des animaux.

Mots-clés : farine de feuilles de neem, profil sanguin de lapines sevrées

*Corresponding author email: egbeyalelt@funaab.edu.ng

Introduction

The increase in human population in Nigeria has been an issue that needs attention on how to ensure food security especially adequate protein intake among the populace. In order to maximize food production and meet protein requirements in Nigeria, viable options need to be explored and evaluated. Ajala and Balogun (2004) reported that rabbit production can be one of the ways of alleviating animal protein deficiency in Nigeria. This is attributed to the immense potentials of these animals including high growth rates, high efficiency in converting forage to meat, short gestation period, high prolificacy, relatively low cost of production and the high nutritional quality of rabbit meat which includes low fat, sodium, and cholesterol levels. Rabbit meat also has a high protein level of about 20.8% and its consumption in Nigeria is bereft of cultural and religious biases (Biobaku and Oguntona, 1997). According to Ghosh *et al*. (2008), rabbit rearing in Nigeria is gaining momentum and this could be attributed to their great potentials. The rabbit has the ability to convert feedstuffs such as forages, most agricultural by-products, kitchen waste etc. that human beings cannot consume directly into highly nutritious meat.

The high cost of conventional feedstuff for livestock has resulted in its scarcity and this is a major problem in developed countries. Due to this there is competition between man and animals for the components of conventional livestock feeds and this necessitates the need for maximizing the effective utilization of non-conventional feedstuffs. This can be achieved by reducing the quantity of expensive feedstuffs and supplementing them with cheaper non-conventional protein feedstuff like neem (*Azadirachta indica*). Neem contains some bioactive compounds that may also alter the haematological and serum biochemical parameters of animals which in turn can influence the growth performance of animals. The neem tree is readily available and always has green leaves all through the year. This study therefore aimed to determine the growth performance, blood profiles and carcass characteristics of weaned female rabbits fed diets containing varying levels of neem leaf meal (NLM).

Materials and Methods

Site of the experiment:

The experiment was carried out at the rabbitry unit of the Teaching and Research Farms, Federal University of Agriculture (FUNAAB), Alabata, Abeokuta, Ogun State, Nigeria. The university is located on latitude 7°10′N, longitude 3°2′E at an altitude of 76m above sea level. It is in the South-Western part of Nigeria and has a tropical climate with a mean annual rainfall of 1,037 mm; an average temperature of 34.7°C and a relative humidity of 82%. The vegetation in the University represents the interphase between the tropical rainforest and the derived savannah (Google Earth, 2017).

Preparation of Neem Leaf Meal:

Fresh Neem (*Azadirachta indica*) leaves were harvested and weighed before air drying The fresh leaves were placed on a polythene sheet to prevent volatile nutrients from escaping through direct sunlight. They were air dried and turned regularly to ensure even drying and to prevent the leaves from decaying during drying. The air dried leaves were milled. The neem leaf meal (NLM) was used at inclusion levels of 0, 5, 10 and 15% to replace wheat offal in the rabbit's diet. The composition of the diets is shown in Table 1.

Experimental Animals and Management:

Thirty-six (36) female weaned rabbits (does) with initial weight ranging from 550 to 700g were used for the experiment. The animals were randomly divided into four treatment groups of nine rabbits per groups. Each treatment group was further divided into three replicates with three rabbits each and on weight equalization they were assigned to four dietary treatments of 0, 5, 10, and 15%NLM in a completely randomized design (CRD) experiment. The animals were housed in wooden hutches that were washed and

Table 1: Percentage Composition of the Experimental Diets

	Inclusion level of NLM (%)			
Ingredients	**0**	**5**	**10**	**15**
Maize	40	40	40	40
Wheat offal	15	10	5	0
Rice husk	17	17	17	17
Neem leaf meal	0	5	10	15
Soybean meal	23	23	23	23
Oyster shell	1.5	1.5	1.5	1.5
Bone meal	3.0	3.0	3.0	3.0
*Vit./premix	0.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated analysis				
Crude protein (%)	18.01	18.10	18.22	18.27
Crude fibre (%)	10.12	10.19	10.22	10.33
Energy (kcal/ kg)	2638.24	2589.84	2541.44	2493.04

**Vit/Min. Premix contained: premix (Embavit No 90) contained Vit A, 10 000 000iu; D3, 2 000 000iu; E, 12 500iu; K, 1.30g; B1, 1.30g; B2, 4.00g; D Calcium – pantothenate, 1.30g; B6, 1.30g; B12, 0.01g; nicotinic acid, 15.00g; folic acid, 0.05g; biotin, 0.02g; Co, 0.20g; Cu, 5.00g; Fe, 25.00g; I, 0.06g; Mn, 48.00g; Se, 0.10g; Zn, 45.00g; choline chloride, 200.00g; BHT, 50.00g.*

disinfected using Morigad one week before the arrival of the rabbits. Water and feed were given to the rabbits *ad libitum* and general sanitation was done, with proper biosecurity and daily routine management.

Data Collection

Growth performance: the feed intake was calculated on a daily basis while the animals were weighed on a weekly basis. The weight gain was determined by subtracting the final weight from the initial weight of each rabbit. The average daily weight gain was calculated by dividing the total weight gain by the period of the experiment.

Collection of Blood Samples:

At the 10th week of the experiment, one rabbit was selected per replicate and blood samples were collected. 2ml of blood was drawn from the ear vein of each animal using a needle and syringe into labeled sterile sample bottles containing ethylene diamine tetra acetate (EDTA) as anti-coagulant for haematological analysis while another 2ml of blood sample was collected into labeled bottles without anticoagulant for serum biochemistry analysis. Haematological indices such as packed cell volume (PCV), Red blood cell (RBC), White blood cell (WBC) and differential leukocyte counts were determined according to the procedures described by (Jain, 1986). Erythrocyte indices (Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated using the appropriate formulae.

- MCV (fL) = PCV (%) x 10 /RBC count (millions/µL)
- MCH (pg) = Hb (g/dL) x 10/RBC count (millions/µL)
- MCHC = Hb (g/dL) x 100/PCV (%) (grams/ dL)

Carcass characteristics:

On the last day of the experiment one rabbit in each replicate was selected and starved for twelve hours, so as to reduce its

gastro-intestinal tract content. The rabbits were weighed and slaughtered and eviscerated. The live weight, carcass weight, dressing weight and weights of the cut parts (Head, Fore-limbs, Hind-limbs, Chest, Loin, Neck, Back, Tail) and visceral organs (liver, kidneys, heart, lungs,) were expressed as the percentage of live weight of each animal.

Statistical Analysis:

Data obtained were subjected to one-way Analysis of Variance in a completely randomized design, using SAS (2003). Significant means were compared using Duncan's Multiple Range Test of the software package.

Results

The effect of neem leaf meal on growth performance of female weaner rabbits is shown in Table 2. The results showed that the animals that fed on the control diet and their counterparts on a dietary inclusion of neem leaf meal at 5% and 10% had similar final live weights and weight gains which were higher than those on 15% dietary inclusion of neem leaf meal. However, the feed conversion ratios were similar across the treatment groups.

The effect of replacing wheat offal with neem leaf meal on the carcass characteristics of female weaner rabbits is presented in Table 3. The results revealed that all carcass cut parts except the head were not influenced ($p>0.05$) by neem leaf meal inclusion. The dressing percentage across the treatment groups ranged from 68.12 to 73.22%. The hind-limbs and backs ranged from 15.30% to 16.80% and 10.84% to 14.24 % respectively.

The haematological parameters of weaned female rabbits fed diets containing varying levels of neem leaf meal are shown in Table 4. The results showed that the replacement of wheat offal with neem leaf meal did not pose any significant ($p>0.05$) influence on all the measured parameters. The PCV mean values ranged from 43.00% to 47.67% while RBC and WBC ranged from 7.13 to 7.97x1012/L and 3.00 to 6.77x109/L, respectively. The results also showed that, there were no significant ($p>0.05$) differences for all parameters measured across the treatment groups (Table 5) in the effects of neem leaf meal on the serum biochemistry of weaned female rabbits.

Table 2: Effect of neem leaf meal on growth performance of female weaner rabbits

	Inclusion level (%) of Neem Leaf Meal			
Parameters	**0**	**5**	**10**	**15**
Initial weight (g)	583.33±9.62	577.78±11.11	577.78±11.11	566.67±0.00
Final weight (g)	1416.67±58.53[a]	1461.11±20.03[a]	1366.67±33.33[a]	1161.11±56.38[b]
Total weight gain (g)	833.33±50.00[a]	833.33±25.46[a]	788.89±29.40[a]	594.44±56.38[b]
Daily weight gain (g)	9.16±0.55[a]	9.71±0.28[a]	8.67±0.32[a]	6.53±0.62[b]
Total feed intake (g)	5383.33±285.99	5396.67±389.40	4905.56±102.89	4755.56±200.54
Daily feed intake (g)	59.16±3.14	59.30±4.28	53.91±1.13	52.26±2.20
Feed conversion ratio	6.50±0.47	6.13±0.54	6.23±0.21	8.23±1.21

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

Table 3: Effect of neem leaf meal on carcass characteristics of female weaner rabbits

	Inclusion level (%) of Neem Leaf Meal			
Parameters	**0**	**5**	**10**	**15**
Live weight (kg)	1568.33±38.43[a]	1594.67±81.98[a]	1368.67 ±38.68[a]	1115.00± 12.87[b]
Carcass weight (g)	1148.67 ± 37.81[a]	1123.00 ±51.64[a]	975.00 ± 56.04[a]	757.67 ± 78.43[b]
Dressing %	73.22 ± 1.09	70.50±0.56	71.17± 2.7	68.12±0.87
Cut part (%)				
Head	9.40± 0.43[a,b]	8.29 ± 0.19[a]	9.97 ± 0.23[a,b]	10.30 ± 0.63[a]
Forelimb	9.08±0.90	6.51± 1.88	7.51± 1.14	8.64 ± 0.43
Hind limb	16.34 ±0.83	15.30± 0.24	16.80± 0.92	15.41 ± 0.40
Chest	11.70± 0.68	13.21± 1.14	13.06 ± 1.8	10.24 ± 0.62
Loin	8.23 ± 1.17	7.90 ± 0.30	6.62 ± 0.31	7.66 ± 0.39
Neck	1.56 ± 0.23	1.95 ± 0.20	2.10 ± 0.15	1.92 ± 0.19
Back	14.24 ± 0.63	13.67 ± 1.43	12.64 ± 0.89	10.84 ± 0.54
Tail	0.81 ± 0.08	0.51 ± 0.09	0.70 ± 0.14	0.54 ± 0.05
Liver	2.11± 0.21	2.50 ± 0.02	2.74 ± 0.25	2.52 ± 0.11
Kidney	0.62 ±0.03	0.51 ± 0.09	0.51 ± 0.03	0.45 ± 0.02
Heart	0.32 ± 0.04	0.25 ± 0.03	0.29 ± 0.03	0.23 ± 0.03
Lung	0.61 ± 0.72	0.50 ± 0.03	0.61 ±0.06	0.57 ± 0.04

[a,b]: Means on the same row with different superscripts are significantly (P<0.05) different

Table 4: Effects of neem leaf meal on haematological parameters of weaned rabbits

	Inclusion level (%) of Neem Leaf Meal			
Parameters	**0**	**5**	**10**	**15**
Pack Cell Volume (%)	45.00±0.58	47.67±3.84	46.00±0.58	43.00±0.58
Haemoglobin (g/dl)	15.00±0.17	15.87±1.27	15.17±0.35	14.40±0.31
Red Blood Cell (×1012/L)	7.77±0.23	7.97±0.65	7.70±0.15	7.13±0.33
White Blood Cell (×109/L)	3.00±0.58	4.50±1.35	5.33±1.20	6.77±1.28
Heterophil (%)	24.67±4.06	32.67±2.19	34.67±2.03	34.00±1.15
Lymphocytes (%)	73.33±4.41	64.67±2.40	64.00±1.73	64.33±0.88
Eosinophil (%)	0.33±0.33	0.67±0.33	0.33±0.33	0.33±0.33
Basophil (%)	1.00±0.00	0.33±0.33	0.33±0.33	0.67±0.33
Monocytes (%)	0.67±0.33	1.67±0.88	0.67±0.33	0.67±0.67
MCV (fL)	58.01±1.19	59.80±0.10	59.76±0.53	60.27±0.85
MCH (pg)	19.33±0.41	19.92±0.04	19.70±0.23	20.19±0.47
MCHC (g/dl)	33.33±0.04	33.29±0.02	32.96±0.37	33.48±0.32

MCV: Mean Cell Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

Table 5: Effects of neem leaf meal on serum biochemical parameters of weaned rabbits

	Inclusion level (%) of Neem Leaf Meal			
Parameters	**0**	**5**	**10**	**15**
Total protein (g/dl)	5.40±0.46	6.27±0.63	5.57±0.87	7.40±0.62
Albumin (g/dl)	2.93±0.09	3.23±0.22	3.07±0.41	4.00±0.36
Globulin (g/dl)	2.47±0.44	3.03±0.42	2.50±0.47	3.40±0.36
Cholesterol (mg/dl)	49.00±0.58	52.33±2.19	52.67±4.18	51.33±0.67
Glucose (mg/dl)	78.00±6.08	91.67±8.41	90.67±9.21	92.00±7.02
Urea (mg/dl)	3.00±0.20	2.80±0.40	2.27±0.18	3.27±0.19
Creatinine (mg/dl)	0.87±0.22	0.70±0.35	0.70±0.12	0.87±0.33
AST (U/L)	44.67±4.41	42.67±4.63	52.33±7.69	42.67±5.61
ALT (U/L)	26.33±1.45	30.33±1.20	29.00±2.00	27.67±4.63
ALP (U/L)	48.33±2.85	50.00±2.08	42.33±2.03	45.33±2.02

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline phosphatase

Discussion

The similarities in the weight gain of rabbits fed on the control diet and their counterparts on diets with 5% and 10% NLM inclusion could have also resulted from the anti-oxidant property of neem (Demiray *et al*., 2009; Ghimeray *et al*., 2009; Olabinri *et al*., 2009) as well as the extra nutrients as reported by Ogbuewu *et al*. (2011) that neem leaves contain appreciable amounts of proteins, minerals, carotene and adequate amount of trace minerals. The reduction in weight gain beyond 10% NLM inclusion agrees with the work of Dagbir *et al*. (1980) who reported that bulkiness of feed makes animals unable to meet their energy and protein requirements. It could also be due to the increased presence of anti-nutritional factors with increase in the quantity of NLM as indicated by Dutta *et al*. (1986). The similarities in the feed conversion ratios across the treatment groups is not in line with the report of Unigwe *et al*. (2016) who used unsexed weaner rabbits on the same test ingredient. The significant effect reported by Unigwe *et al*. (2016) could be the masked effect of male rabbit responses to the dietary inclusion of neem leaf meal.

The decrease in carcass weight obtained as the dietary levels of neem leaf meal increased is relative to the final live weight of the animals before slaughter. The similarities in the values of the livers across the treatments could be evidence of the hepatoprotective nature of the neem leaf (Chattopadhyay *et al*., 1992). This also may reflect the ability of rabbits to adequately handle and tolerates anti nutritional factors at these dietary inclusion levels of neem leaf meal. This capacity is meditated through coprophagy and caecal fermentation

The haematological values obtained in this study, are within the standard range recommended for clinically healthy rabbits (Ogbuewu *et al*., 2010). The similarities in red blood cells (RBC), packed cell volume (PCV) and haemoglobin (Hb) observed for the rabbits on NLM diets relative to the control group is an indication that the animals were not anemic and this implies that up to 15 % inclusion level of NLM does not have a detrimental effect on the relative quantity of blood cells as well as the total volume of blood. The range of MCHC obtained in the present study is within the range of 31.1-37.0% reported by Mitruka and Rawnsley (1977) as cited by (Ogbuewu *et al*., 2010) for clinically healthy rabbits. Thompson (2006) reported that MCHC values have been shown to be the most accurate and absolute values that indicate an anemic condition in animals. The MCV values observed in this study were within the normal range of 58.0-79.6 fL (femtoliters) reported for healthy rabbits

(Mitruka and Rawnsley, 1977). The MCH values of all treatment groups were within the range of 19.2 × 10-12 to 29.5 × 10-12 g reported for healthy rabbits by Mitruka and Rawnsley (1977).

The similarities observed in total proteins in the present study are in accordance with the earlier report on protein retained in animals (Akinola and Abiola, 1999). Iyayi and Tewe (1998) and Awosanya *et al*. (2000) reported the dependence of blood proteins and creatinine on the quality and quantity of dietary proteins. Similarities in the serum glucose levels in the present study could imply that bioactive compounds contained in neem leaves which have the ability to block the energy metabolic pathway (Chattopadhyay, 1996), were not present in sufficiently high concentrations to make it difficult for the animals to meet their energy requirements. This however, is in contrast to the findings of Ogbuewu *et al*. (2009) who reported a decrease in the serum glucose of male rabbits using the sam*E inclusion* levels of neem leaves as utilized in the present study. The comparable treatment values further imply that the rabbits were in a state of normal nitrogen balance. The comparable values in the serum cholesterol levels of rabbits fed neem leaf to the control probably suggests a normality in lipid mobilization (Ogbuewu *et al*., 2009). The similarities in all the haematological and serum biochemical indices observed in this study were at variance with the findings of Ogbuewu *et al*. (2009). This could be as a result of the different sexes used for the study although the level of neem leaf meal inclusions was the same.

Conclusion

The study concluded that replacement of wheat offal with neem leaf meal should not exceed 10% inclusion levels in the diet of female weaner rabbits to prevent poor weight gain.

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AFRICAN UNION - INTERAFRICAN B*UREA*U FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
September 2019

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican B*urea*u 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.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

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- Marketing, economics
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The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese), by the editors, after acceptance. Full articles submitted in French will also be published in English.

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- Experiments, statistics, and other analyses performed are described in sufficient detail. The research must have been performed to a technical standard to allow robust conclusions to be drawn from the data. Methods and reagents must also be described in sufficient detail so that another researcher is able to reproduce the experiments described.
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Obituary articles to honor prominent African scientists that have made significant contribution to animal resources research and development

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Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

1. On cover page of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, , title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided..
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-repetitive way. Subheadings may be accepted.
8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. . Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
10. State the conclusions, and any implications that may be drawn from the study.

Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double line spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
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- *Journal Articles:* Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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