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BLOOD SERUM AND EGG *LIPOPROTEIN*S OF LAYING BIRDS ADMINISTERED EXTRACT OF *LAGENARIA BREVIFLORA R.* UNDERTWO MANAGEMENT SYSTEMS

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Abstract

The continuous demand of the ever-growing Nigerian populace to meet their daily protein requirement has resulted in a positive shift in the consumption of table eggs but with many opting for alternatives because of the perceived *Cholesterol* content in eggs. This study was therefore carried out to investigate the potential effect of *Lagenaria breviflora R.*on the blood and egg *Lipoprotein*s of laying birds under two management systems. A total of 126 yaffa brown layers aged 36 weeks were administered extracts of *Lagenaria breviflora R.* at 3 levels (control (0), 200 and 300 g fresh weight per 4 litres of water) under two management systems (Deep litter 'DL' and Deep litter with a run 'DLR'). Blood and egg samples were collected at 0, 5th and 10th weeks. Data obtained on blood and egg lipid profiles (initial, mid, final, difference and percent change) were arranged in a 2 x 3 factorial layout in a completely randomized design. The percentage reduction (P<0.0.5) in the blood total *Cholesterol* (TC) was higher in the 300 g dosed group. The blood high density *Lipoprotein* (HDL), differential and percentage change (reduction) values of blood low density *Lipoprotein* (LDL) were highest (P<0.05) in the 200 g dosed group. The TC difference and percent change (reduction) were significantly higher (P<0.05) in DL raised birds. Birds raised on the DLR management system had an improved HDL. The yolk TC differential and percent change (decrease) were lowest (P<0.05) in the 200 g dosed group. The yolk LDL difference and percent change (reduction) were highest in the 200 g dosed treatment group. Management systems had influence (P<0.05) on most yolk *Lipoprotein* parameters measured except TC, LDL and VLDL. The mid, final, differential and percent change (increase) in HDL were higher (P<0.05) in eggs sampled from birds raised on the DLR management system. The interactive effect of both factors on LDL revealed positive (P<0.05) differences and percent change in values in all treatment groups with the highest reduction observed in the DL (Control) group. The reduction in TC was highest in DL (200 g) compared to other treatment groups. The yolk HDL-*Cholesterol* percent change was highest (P<0.05) while the LDL was lower in eggs sampled from birds raised on DL and administered 200 g LB. Therefore, commercial layers can be raised on the DLR system and administered LB up to 200 g as an alternative medication in *Cholesterol* reduction in poultry products with no adverse health effects.

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Introduction

Poultry products (meat and eggs) are considered as a good and low-priced source of animal protein because of their good and high biological value. Therefore, it plays a significant role in human diet in meeting their daily protein requirements. There has been a growing concern in recent years over the concentration of *Cholesterol* in the human diet, since eggs are a concentrated source of *Cholesterol* in the diet and limited egg consumption has often been recommended to reduce total serum *Cholesterol* concentrations. Chicken eggs haves been recognized as an excellent source of all essential nutrients and a complete food for people of all ages (Salma *et al*., 2007). Many reviews have postulated the relationship between yolk *Cholesterol* and cardiovascular diseases in humans but with no clear association between egg consumption and cardiopathies, eggs can be part of an overall heart-healthy diet (Eckel, 2008; Wang *et al*., 2009).

Cholesterol is a fatty substance existing in all the cells in the body and is therefore present in animal foods. *Cholesterol* travels in the blood as particles called *Lipoprotein*s and is an important constituent of cell membranes modulating their fluidity; it is a precursor of steroid hormones (Kasprzak and Hetmañski, 2004). Total *Cholesterol* is made up of low-density *Lipoprotein*s (LDL), high-density *Lipoprotein*s (HDL) and very low density *Lipoprotein*s (VLDL).

There are two known facts that *Cholesterol* must be in the rancid form to cause the arterial plaques that lead to partial blockage of the blood vessels while some forms of *Cholesterol* are beneficial to man. High-density *Lipoprotein Cholesterol* (HDL) has been established to protect against heart disease by mopping up circulating *Cholesterol* units in the system (Narahari, 2003). The culprit responsible for this oxidized form that narrows or "hardens" the arteries is the low-density *Lipoprotein Cholesterol* (LDL) and low levels of the HDL fraction (Chandler *et al*., 1979; Kasprzak and Hetmañski, 2004). Therefore, it is imperative to counteract this process by eating foods rich in natural antioxidants. Researchers have focused on the beneficial effects of phytogenic substances in poultry production. These phytogenic substances contain Phenolic compounds that have been documented to have hypo-*Cholesterol*emic effects: *Cholesterol* and *Lipoprotein* decreasing effects of alfalfa (Ponte *et al*., 2004), thyme (Bolukbasi *et al*., 2006) and garlic (Habibian Dehkordi *et al*., 2010). There is an urgent need to increase livestock production to meet the animal protein needs of the world's increasing population while making efforts to reduce its *Cholesterol* component. Therefore, the current experiment was designed to study the effects of the administration of extracts of *Lagenaria breviflora* and management systems on *Cholesterol* content in the eggs and blood of laying birds.

Materials and Method

Area depiction

The experiment was carried out at the Poultry unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria located on latitude 7° 15'N, longitude 3° 26' E and 76m above sea level (Google Earth, 2014).

Experimental birds and management

A total of 126 Yaffa brown layers aged 36 weeks were used for the study. The experiment was arranged in a 3 x 2 factorial experimental layout. Variations due to management systems (Deep litter with a run and Deep Litter) and *Lagenaria breviflora* (at 3 levels of administration: 0, 200 and 300 g fresh weight per 4 litres of water): control (where *Lagenaria breviflora* was not given to the birds, but other conventional vaccinations and medications were given). Fresh L. breviflora whole fruit was weighed, cut into pieces, and soaked in 4 L of fresh clean water for 24 h (w/v). Extracts were administered to birds daily over the duration of the experiment.

The birds were randomly allotted into six treatment groups. Each treatment group was assigned twenty-one (21) birds and these were further divided into three replicates of

seven (7) birds each. The experiment was carried out for a period of ten (10) weeks. Birds were provided with commercial layers' mash diet containing 17.10% CP and 2709 Kcal/Kg ME layers mash. The pullets were provided with nest boxes.

Collection of Blood Samples

Blood samples were collected very early in the morning from three tagged birds from each replicate via venipuncture of the wing vein into bottles. The blood was allowed to clot by leaving it undisturbed at room temperature for 30 minutes. The clot was separated from the serum by centrifuging for 10 minutes (indicate speed)?. The sera were tested in the laboratory to determine the total *Cholesterol*, high density *Lipoprotein* (HDL), low density *Lipoprotein* (LDL) and very low *Lipoprotein* (VLDL) contents. The blood samples were collected three times over the duration of the experiment. The blood collection periods were at the start of the study, mid-way (5weeks) and at the end (10 weeks) of the experiment.

Collection of Egg Sample

Five egg samples collected from each replicate were tested to determine the total *Cholesterol*, high density *Lipoprotein* (HDL), low density *Lipoprotein* (LDL), and very low density *Lipoprotein* (VLDL) contents. The egg samples were collected at the same intervals as the blood samples and the yolk was analyzed for *Cholesterol* and *Lipoprotein* content.

Determination of Total Cholesterol

Serum *Cholesterol* was determined spectrophotometrically according to the methods of Allain *et al*, (1974). The reagent was made up of three enzymes, *Cholesterol* esterase (CE) *Cholesterol* oxidase (CO) and peroxidase (POD), and two substrates; 4-aminoantipyrine (4-AA) and phenol.

Procedure:

Three clean test tubes labeled blank (B), standard (S), and test (T) were arranged in a test tube rack. 10ml of distilled water, standard *Cholesterol* and serum was added to each of the test tubes respectively. 1ml of the reagent was then added to each of the test tubes. After mixing, the reaction mixtures were incubated for 10minutes at ambient temperature. The absorbance of the standard *Cholesterol* and the serum samples was read at 550mm wavelength against the reagent blank.

Determination of HDL

$$\text{Cholesterol Concentration in Serum } \left(\frac{\text{mg}}{\text{dl}}\right) = \frac{(\text{Absorbance of test} \times \text{Concentration Of STD})}{\text{Absorbance of STD}}$$

This was determined using the method of Grillo *et al*. (1981). Three clean test tubes marked and labeled as blank (B), standard (S) and test (T) were arranged in a test tube rack. To each of these tubes were added 1.0 ml of working reagent while 0.05 ml distilled water, HDL standard and super nutrient was added to each test tube respectively. The reaction mixtures were incubated at 37 0c for 5 minutes. The absorbance of standard (Abs. S) and Test sample (Abs. T) against the blank were measured within 60 minutes using a spectrophotometer.

Calculation:

$$HDL\ cholesterol\ (mgldl) = \frac{Abs.T \times Con.Of\ STD}{Abs.S}$$

Determination of LDL-C

The *Triglycerides*' content was determined using Friedewald's formulae (1972). The concentration of very low density *Lipoprotein* (VLDL) *Cholesterol* was therefore estimated using.

$$LDL - cholesterol = Total\ Cholesterol - HDL - \frac{triglyceride}{5}$$

Determination of VLDL-Cholesterol

The concentration of very low density *Lipoprotein* (VLDL) *Cholesterol* was calculated by modification of the Freidwald's formulae. *Triglyceride*s were determined using standard methods (Fossati and Prencipe, 1982) and used in the estimation of VLDL.

$$VLDL - Cholesterol = triglyceride\ value\ divide\ by\ 5$$

Statistical Analysis

Data obtained were arranged in a 2 x 3 factorial experimental layout and analyzed using the *General*ized Linear Model (GLM) of IBM SPSS Statistics 20.0 (2011). The initial values obtained (egg and blood *Lipoprotein*) were used as covariates. Significant differences among the treatment means were determined at 5% level of significance.

Statistical Model

$$\gamma_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \square_l + \varepsilon_{ijk}$$

Where,

γ_{ijk} = Observed value of the dependent variable (output)

μ = Population mean (Overall mean)

τ_i = Effect of Management systems (i = Deep Litter with a run, Deep Litter)

β_j = Effect of *Lagenaria breviflora R.*administration (0, 200 and 300g/4litres of water)

$(\tau\beta)_{ij}$ = Effects of interaction between Management systems and *Lagenaria breviflora R.* administration

$\square_l$ = Covariate effect of initial values for each parameter (Total *Cholesterol*, HDL, LDL and VLDL)

ε_{ijk} = Random residual error.

Results

Effect of Lagenaria breviflora extract administration on blood and egg Cholesterol

Blood Lipids

The effect of *Lagenaria breviflora* administration on the blood lipid profile of birds is presented in Table 1. The mid and final Total *Cholesterol* (TC) was highest (P<0.05) in the 200 g dosed group with the least obtained in the control and 300 g-dosed groups. The percentage change in total *Cholesterol* for doses of 200 g was statistically lowest (P<0.05) compared to 300 g / 4litres of *Lagenaria breviflora* extract and the control groups.

The percentage change (increase) in HDL-*Cholesterol* was highest (P<0.05) in the 200g dosed group while a reduction was observed in birds in the control (0 g) and 300 g of *Lagenaria breviflora*/4 litres of water groups. The percentage reduction in VLDL was significantly influenced (P<0.05) by the level of administration of *Lagenaria breviflora* extract. The percentage reduction was dose-dependent with the highest value obtained in birds administered 300 g/4litres of water which was statistically comparable to the control while the least was obtained in the 200 g-dosed birds.

A continuous reduction in LDL values (mid and final) (P<0.05) was observed in all treatment groups. However, the difference and percentage change (reduction) values were highest (P<0.05) in birds administered 200 g/4 litres *Lagenaria breviflora* extract.

Egg Cholesterol

The effect of *Lagenaria breviflora* administration on egg-yolk *Lipoproteins* is presented in Table 2. There was a negative change in Total *Cholesterol* in 0 g and 300 g/4 litres LB administered treatment groups while a positive change indicating a reduction was observed in eggs sampled in the 200 g/4 litres treatment group. The Total *Cholesterol* differential and percent change (decrease) was lowest (P<0.05) in the 200 g/4 litres dosed group while an increase was observed in eggs sampled from birds in the control and 300 g/4 litres groups.

There was a decrease in the percent change of HDL component as the level of administration of *Lagenaria breviflora* extract increased from control to 300 g per 4 litres of water. Only the percent change (increase) in yolk HDL was significantly affected by *Lagenaria breviflora* extract administration, the highest percent increase was statistically similar in 0 and 200 g/ 4 litres treatment groups while the least was obtained in 300 g/4 litres dosed group.

Yolk mid and final LDL values were statistically highest and lowest in the 200 and 300 g/4 litres dosed groups respectively. However, the LDL difference and percent change (reduction) was highest in 200 g/4 litres dosed treatment group. There was a decrease in the percent change of HDL component as

the level of administration of *Lagenaria breviflora* extract increased from control to 300 g per 4 litres of water. The percentage change in the VLDL component of eggs was positive in all the treatment groups indicating a decrease with the least change observed in the 300 g/4litres dosed birds. Egg-yolk mid and final VLDL was similar (P<0.05) and lowest in the control and the 200 g/4 litres dosed group with the highest value obtained in 300 g/4litres dosed group.

Effect of Management system on blood and egg Cholesterol

Blood

The effect of the level of administration of *Lagenaria breviflora* on yolk *Lipoprotein*s is presented in Table 1. There was a gradual reduction in the total *Cholesterol* inblood from mid to the end of the experiment in both management systems. Total *Cholesterol* (TC) value was highest (P<0.05) in the mid and final values in birds raised on DLR compared to DL. The TC difference and percent change (reduction) was significantly highest (P<0.05) in DL raised birds.The highest percent change was observed in birds raised on DL. Birds raised on the DLR management system had an improved HDL while a reduction was observed in birds on DL.

Birds managed on both management systems experienced a reduction in LDL. Birds on the DLR management system had higher mid and final values while higher differential and percent changes (reduction) were obtained in birds on DL. The percent change in the VLDL component of blood was statistically similar in both management systems.

Egg

The effect of management systems on egg-yolk *Lipoprotein*s is presented in Table 2. The management system had influence (P<0.05) on most parameters measured except total *Cholesterol*, LDL and VLDL. The mid, final, differential and percent change (increase) in HDL was higher (P<0.05) in eggs sampled from birds raised on the DLR management system than on the DL system.

Interactive effects of Lagenaria breviflora R. and production systems on blood Lipoprotein profile

The interactive effect of both factors presented on Table 3, revealed higher differences and percent change (TC) in the control groups (DL, DLR). Nonetheless, birds on 300 g LB/ 4 litres (DL and DLR) groups experienced more reduction in TC¬¬¬ value compared to that obtained in 200 g LB (DL, DLR) groups. The difference (increase) in HDL value was highest (P<0.05) in birds administered 200 g LB on both management system followed by birds on 300 g/ 4 litres LB (DLR) with other groups experiencing a decrease.The interactive effect of both factors on LDL revealed positive (P<0.05) difference and percent change values in all treatment groups with the highest reduction observed in DL (Control) and followed by DLR (200 and 300 g/ 4 litres LB). The blood LDL content was highest (P<0.05) in DLR (control) both in mid and final values. The blood VLDL content (difference and percent change) was negative (P<0.05) in DL (200 g/ 4 litres LB) indicating an increase in value, with the highest reduction in DL (300 g/ 4 litres LB) and DLR (control).

The interactive effect of Lagenaria breviflora R. and production systems on egg Lipoprotein profile

The interactive effect of *Lagenaria breviflora R.* and production systems on egg *Lipoprotein* profile is shown in Table 4. The percent change and difference in TC content of eggs was negative in all treatment groups except DLR (200 g/4 litres). The reduction in TC was highest in DL (200 g/4 litres) compared to other treatment groups.The HDL *Cholesterol* percent change in sampled eggs was least (P<0.05) in eggs sampled from birds raised on DL and administered 300 g/4 litres LB. LDL-*Cholesterol*, all treatment groups experienced decreases in value throughout the data collection period except birds on DL (300 g/4 litres LB) and DLR (300 g/4 litres LB) which had an increase as indicated in the differential and percent change values.The highest reduction in LDL was observed in DL (200 g/4 litres LB).

Table 1: Effects of *Lagenaria breviflora R.* administration and management systems on blood *Lipoproteins* of laying birds

		Lagenaria breviflora R.					**Management Systems**			
							Control			
		(0g)	**200**	**300**	**SEM**	**P-Value**	**DL**	**DLR**	**SEM**	**P-Value**
Total *Cholesterol* (mg/dl)	Mid	155.07^{b}	194.20^{a}	155.48^{c}	9.06	0.0001	164.53^{b}	171.97^{a}	1.55	0.058
	Final	136.30^{c}	154.32^{a}	148.38^{b}	8.40	0.0001	140.56^{b}	152.11^{a}	1.44	0.009
	Difference	49.16	8.58^{c}	31.22^{b}	7.65	0.0001	36.97^{a}	22.34^{b}	1.31	0.002
	Percent Change	20.36^{a}	3.05^{b}	17.59^{a}	4.37	0.0001	19.46^{a}	11.86^{b}	0.75	0.004
High Density *Lipoprotein* (mg/dl)	Mid	51.42	43.79	48.29	2.90	0.514	50.25	45.42	0.65	0.011
	Final	38.67^{c}	49.04^{a}	44.79^{b}	1.30	0.0001	42.92^{b}	45.42^{a}	0.29	0.006
	Difference	8.54^{a}	-6.96^{c}	0.70^{b}	0.96	0.001	2.33^{a}	-0.81^{b}	0.21	0.001
	Percent Change	15.26^{a}	-19.08^{c}	1.00^{b}	2.36	0.002	2.49^{a}	-4.37^{b}	0.53	0.001
Low Density *Lipoprotein* (mg/dl)	Mid	91.00^{a}	77.75^{c}	83.00^{b}	4.05	0.0001	79.00^{b}	88.83^{a}	0.50	0.0001
	Final	71.25^{b}	60.25^{c}	77.00^{a}	8.12	0.0001	64.67^{b}	74.33^{a}	1.00	0.004
	Difference	23.72^{b}	34.31^{a}	17.31^{c}	5.35	0.0001	29.96^{a}	20.27^{b}	0.66	0.001
	Percent Change	23.14^{b}	37.78^{a}	17.83^{c}	5.44	0.0001	30.82^{a}	21.69^{b}	0.67	0.001
Very Low Density *Lipoprotein* (mg/dl)	Mid	31.86^{b}	45.53^{a}	32.11^{b}	2.70	0.0001	37.78	35.22	0.67	0.1000
	Final	30.56^{b}	38.39^{a}	28.81^{b}	2.89	0.0001	33.31	31.86	0.75	0.3100
	Difference	9.85^{a}	-6.87^{b}	8.36^{a}	1.93	0.0001	3.75	3.81	0.48	0.95
	Percent Change	24.50^{a}	-24.45^{b}	22.14^{a}	5.91	0.0001	7.00	7.80	1.46	0.79

$^{a, b, c}$ Means in the same row by factor with different superscripts differ significantly(P<0.05)
SEM: Standard Error of Mean Difference = Initial – Final Percent Change= (Difference/Initial) x 100
Negative value indicate an increase Positive Value indicate a decrease

Table 2: Effects of *Lagenaria breviflora R.* administration and Management systems on egg *Lipoproteins*

Management Systems		**DL**			**DLR**				
Lagenaria breviflora R.		**0**	**200**	**300**	**0**	**200**	**300**	**SEM**	**P-Value**
Parameters									
Total *Cholesterol* (mg/dl)	Mid	144.01^{c}	191.18^{a}	158.40^{bc}	166.12^{b}	197.23^{a}	152.57^{c}	9.01	0.0001
	Final	122.19^{b}	155.02^{a}	144.46^{ab}	150.41^{a}	153.63^{a}	152.30^{a}	8.42	0.0001
	Difference	65.57^{a}	9.59^{d}	35.75^{b}	32.75^{b}	7.58^{d}	26.69^{c}	7.67	0.0001
	Percent Change (%)	34.38^{a}	3.97^{d}	20.04^{b}	18.33^{bc}	2.12^{d}	15.14^{c}	4.38	0.0001
High Density *Lipoprotein* (mg/dl)	Mid	57.17^{a}	42.42^{c}	51.17^{a}	45.67^{b}	45.17^{b}	45.42^{b}	2.97	0.0001
	Final	40.17^{b}	48.92^{a}	39.67^{b}	37.17^{c}	49.17^{a}	49.92^{a}	1.33	0.0001
	Difference	7.25^{b}	-6.44e	6.17^{c}	9.83^{a}	-7.47^{e}	-4.78^{d}	0.98	0.0001
	Percent Change (%)	12.02^{b}	-17.20d	12.63^{b}	18.50^{a}	-20.96^{e}	-10.64^{c}	2.47	0.0001
Low Density *Lipoprotein* (mg/dl)	Mid	74.00^{c}	76.00c	87.00^{b}	108.00^{a}	79.50^{b}	79.00^{b}	4.06	0.0001
	Final	52.50^{c}	60.00c	81.50^{b}	90.00^{a}	60.50^{c}	72.50^{b}	8.12	0.0001
	Difference	42.15^{a}	34.93a	12.79^{d}	5.28^{e}	33.68^{a}	21.83^{c}	5.36	0.0001
	Percent Change (%)	40.52^{a}	38.52a	13.40^{d}	5.76^{e}	37.04^{a}	22.25^{c}	5.45	0.0001
Very Low Density *Lipoprotein* (mg/dl)	Mid	36.28^{b}	48.78a	28.28^{c}	27.44^{c}	42.28^{a}	35.94^{b}	2.77	0.0001
	Final	34.14^{b}	40.64a	25.14^{c}	26.97^{c}	36.14^{b}	32.47^{bc}	2.86	0.0001
	Difference	7.11^{b}	-8.76e	12.89^{a}	12.58^{a}	-4.98^{d}	3.83^{c}	1.99	0.0001
	Percent Change (%)	17.55^{b}	-30.32d	33.76^{a}	31.44^{a}	-18.57^{c}	10.52^{b}	6.07	0.0001

$^{a, b, c}$: Means in the same row by factor with different superscripts differ significantly(P<0.05) SEM: Standard Error of Mean
Difference = Initial – Final Percent Change= (Difference/Initial) x 100 Negative value indicate an increase
Positive Value indicate a decrease

Table 3: Interactive effects of *Lagenaria breviflora R.* administration and management systems on blood *Lipoproteins* of laying birds

Management Systems		**DL**			**DLR**				
Lagenaria breviflora R.		**0**	**200**	**300**	**0**	**200**	**300**	**SEM**	**P-Value**
Parameters									
Total *Cholesterol* (mg/dl)	Mid	144.01^{c}	191.18^{a}	158.40^{bc}	166.12^{b}	197.23^{a}	152.57^{c}	9.01	0.0001
	Final	122.19^{b}	155.02^{a}	144.46^{ab}	150.41^{a}	153.63^{a}	152.30^{a}	8.42	0.0001
	Difference	65.57^{a}	9.59^{d}	35.75^{b}	32.75^{b}	7.58^{d}	26.69^{c}	7.67	0.0001
	Percent Change (%)	34.38^{a}	3.97^{d}	20.04^{b}	18.33^{bc}	2.12^{d}	15.14^{c}	4.38	0.0001
High Density *Lipoprotein* (mg/dl)	Mid	57.17^{a}	42.42^{c}	51.17^{a}	45.67^{b}	45.17^{b}	45.42^{b}	2.97	0.0001
	Final	40.17^{b}	48.92^{a}	39.67^{b}	37.17^{c}	49.17^{a}	49.92^{a}	1.33	0.0001
	Difference	7.25^{b}	-6.44^{e}	6.17^{c}	9.83^{a}	-7.47^{e}	-4.78^{d}	0.98	0.0001
	Percent Change (%)	12.02^{b}	-17.20^{d}	12.63^{b}	18.50^{a}	-20.96^{e}	-10.64^{c}	2.47	0.0001
Low Density *Lipoprotein* (mg/dl)	Mid	74.00^{c}	76.00^{c}	87.00^{b}	108.00^{a}	79.50^{b}	79.00^{b}	4.06	0.0001
	Final	52.50^{c}	60.00^{c}	81.50^{b}	90.00^{a}	60.50^{c}	72.50^{b}	8.12	0.0001
	Difference	42.15^{a}	34.93^{a}	12.79^{d}	5.28^{e}	33.68^{a}	21.83^{c}	5.36	0.0001
	Percent Change (%)	40.52^{a}	38.52^{a}	13.40^{d}	5.76^{e}	37.04^{a}	22.25^{c}	5.45	0.0001
Very Low Density *Lipoprotein* (mg/dl)	Mid	36.28^{b}	48.78^{a}	28.28^{c}	27.44^{c}	42.28^{a}	35.94^{b}	2.77	0.0001
	Final	34.14^{b}	40.64^{a}	25.14^{c}	26.97^{c}	36.14^{b}	32.47^{bc}	2.86	0.0001
	Difference	7.11^{b}	-8.76^{e}	12.89^{a}	12.58^{a}	-4.98^{d}	3.83^{c}	1.99	0.0001
	Percent Change (%)	17.55^{b}	-30.32^{d}	33.76^{a}	31.44^{a}	-18.57^{c}	10.52^{b}	6.07	0.0001

$^{a, b, c, d, e}$: Means in the same row by factor with different superscripts differ significantly(P<0.05)
SEM: Standard Error of Mean Difference = Initial – Final Percent Change= (Difference/Initial) x 100
Negative value indicate an increase Positive Value indicate a decrease

The difference in VLDL was highest in eggs sampled from birds managed on DLR (control) the least change in DL (300 g/4 litres LB) while the highest (P<0.05) in DL (200 g/4 litres LB) and DLR (control).

Discussion

The percentage reduction in Total *Cholesterol* was higher in 300 g/ 4 litres LB dosed group. This was more comparable to the control group than the 200 g/4 litres) treatment group. This can be ascribed to its sulphur containing compounds which are capable of reacting with the SH group systems (Prasad *et al*., 2009). *Lagenaria breviflora R.* has been established to comprise of compounds like higher hydrocarbons (higher alkanes, alkenes, benzenes) and sulphur-containing ones amongst them is 2-methyl-Benzothiazole. This is made possible by the mechanism of hypo-*Cholesterol*aemic and hypo-lipidemic action of the SH-group which is known to be proficient in altering the hepatic activities of lipogenic and cholesterogenic enzymes thereby significantly reducing *Cholesterol* content in the blood of birds especially when administered at higher dosages.

There was a sharp reduction in the blood HDL-*Cholesterol* value as the birds aged with the highest percentage change observed in the control group. HDL-*Cholesterol* was greatly favoured in the 200 g/4 litres dosed birds. This affirms the ability of LB to favour the redistribution of *Cholesterol* amongst *Lipoprotein* molecules by lower LDL-*Cholesterol* levels. The higher phenolic components of LB may be responsible for the anti-atherogenic activities exhibited through maintenance/ slight reduction of HDL-*Cholesterol* resulting in and prevention of *genera*tion of oxidatively modified LDL (Yokozawa *et al*., 2006) as observed in the study since the presence of large HDL particlecorrelates with better health condition whereas the presence of small HDL particles is associated with atheromatous

Table 4: Interactive effect of *Lagenaria breviflora R.* administration and management systems on egg *Lipoproteins*

Management Systems		**DL**			**DLR**				
Lagenaria breviflora R.		**0**	**200**	**300**	**0**	**200**	**300**	**SEM**	**P-Value**
Parameters									
Total *Cholesterol* (mg/dl)	Mid	1718.74	2236.65	685.35	1082.94	1587.12	305.21	512.38	0.7400
	Final	1302.12^{c}	1225.32^{d}	1401.18^{b}	1298.97^{d}	1314.56^{c}	1425.35^{a}	46.72	0.0001
	Difference	-1.18^{b}	88.38^{a}	-128.68^{e}	-17.73^{c}	-22.48^{d}	-161.73^{f}	45.96	0.0001
	Percent Change (%)	-0.65^{b}	5.05^{a}	-10.07^{c}	-1.16^{b}	-1.93^{b}	-13.04^{c}	3.64	0.0001
High Density *Lipoprotein* (mg/dl)	Mid	227.61	244.11	264.94	240.11	261.94	276.78	21.92	0.0530
	Final	340.42^{a}	324.42^{a}	291.92^{b}	347.92^{a}	343.92^{a}	341.92^{a}	8.39	0.0001
	Difference	-101.27	-75.22	-21.32	-111.67	-99.07	-87.07	10.30	0.0001
	Percent Change (%)	-41.84^{c}	-30.20^{b}	-11.76^{a}	-46.98^{c}	-40.25^{b}	-34.59^{b}	4.19	0.0001
Low Density *Lipoprotein* (mg/dl)	Mid	1038.97^{a}	1131.81^{a}	296.47^{d}	790.64^{c}	968.97^{b}	297.14^{d}	161.52	0.0001
	Final	650.44^{c}	586.11^{d}	937.94^{a}	729.78^{b}	706.94^{b}	897.28^{a}	29.59	0.0001
	Difference	112.02^{b}	172.04^{a}	-134.83^{e}	50.70^{c}	66.67^{bc}	-97.50^{d}	47.62c	0.0001
	Percent Change (%)	11.27^{b}	17.25^{a}	-18.13^{d}	6.63^{c}	7.60^{c}	-12.34^{d}	6.32c	0.0001
Very Low Density *Lipoprotein* (mg/dl)	Mid	266.67^{a}	250.00^{ab}	254.50^{ab}	246.17^{b}	260.83^{a}	272.33^{a}	9.84	0.0010
	Final	251.58^{a}	239.25^{b}	248.75^{a}	235.08^{b}	245.42^{a}	249.42^{a}	3.82	0.0001
	Difference	7.78^{e}	18.90^{b}	9.00^{de}	22.98^{a}	13.37^{c}	10.62^{d}	3.29	0.0001
	Percent Change (%)	2.94^{c}	7.06^{a}	3.35^{c}	8.78^{a}	5.15^{b}	3.75^{c}	1.28	0.0001

$^{a, b, c, d, e}$: Means in the same row by factor with different superscripts differ significantly(P<0.05)
Difference = Initial – Final Percent Change= (Difference/Initial) x 100
Negative value indicate an increase Positive Value indicate a decrease

disease progression in arteries.

The mechanism of action of VLDL-*Cholesterol* lowering by *Lagenaria breviflora R.* in the blood of birds may be ambiguous to elucidate.This ability is probably due to LB being capable of influencing lipid profiles (VLDL) through an increase in esterified *Cholesterol* leading to either an increase or reduction in the excretion of esterified *Cholesterol* by bile. These influxes of reactions invariably lower the VLDL-*Cholesterol* as observed by the highest reduction which was dose-dependent. In this regard, the reduction or positive activity of LB on VLDL can be a useful factor in regulating the degree of fatness in birds since lowered VLDL causes a decreased abdominal fat pad in chickens (Tohala, 2010) and also in determining the egg laying status of birds (Peebles *et al*., 2004). Many plant species have been used to alter levels of some markers of disease conditions in order to improve the health status of animals (Ojiako and Nwanjo, 2009; Owen *et al*., 2011). *Lagenaria breviflora R.* (LB) contains phytochemicals notably phenols, carotenoids and flavonoids which have been reported to have a wide orbit of biological activities. The positive influence confirms the mechanism of antioxidant and anti-peroxide action of LB resulting in decrease in hepatic production of VLDL-*Cholesterol* which serves as precursor of LDL-*Cholesterol* in blood circulation (Prasad *et al*., 2009). Onasanwo *et al*. (2011) observed LB to exhibit powerful antioxidant activity capable of significantly inhibiting free radical and superoxide anion (oxidative stress). These phytochemicals have been established to work synergistically in tissues of animal to establish a vital antioxidant defense mechanism against reactive oxygen species (ROS) mediated lipid-peroxidation of *Lipoprotein*s (Adaramoye *et al*., 2005; Celik *et al*., 2011). Hyper*Cholesterol*emia has been linked with oxidative stress caused by an increase in lipid peroxidation resulting in increased LDL (a prerequisite for most forms of atherosclerosis) (Adaramoye *et al*., 2005; Owen *et al*., 2011).

This study should be considered as a first pilot study showing the influence of different doses of the oral administration of *Lagenaria breviflora* in combination with management systems on the blood *Lipoprotein* of laying birds. The increase in the percent change of HDL component at 200 g/4 litres level of administration of *Lagenaria breviflora* extract under both management systems which also corresponds with decreases in the LDL components affirms the ability of *Lagenaria breviflora* to alter the bird's system to reduce the level of LDL in the blood and maintaining an increase in HDL components which is termed a good *Cholesterol*. However, the wide range in change of HDL content observed in the 200 g/4 litres dosed group (DL and DLR) clearly shows the ability of LB in increasing blood HDL content as it was lowest at start of experiment.

The management system used in the study comprised of a deep litter and a modified deep litter with a run. Blood sampled from birds on DL system had higher *Cholesterol* percent change (decrease) while HDL-*Cholesterol* was favoured in the DLR management system. This shows that the extra movement on the run by birds on DLR stimulates the body in producing more TC to cope with the rigours associated with expressing their natural behaviours.

The metabolism of the DLR layers might be faster than the DL layers because of the extra movement in the run coupled with the anti-*Cholesterol*emic ability of LB. This was evidenced in birds administered the highest dosage (300 g/4 litres) in the study and raised on DLR showing improved blood HDL value, reduced LDL and TC. It could be assumed that more movement consumes more energy, and cell construction and digestion used more *Cholesterol* than the amount synthesized by the system. The observed trend in DL (200 g/4 litres) and DLR (200 g/4 litres) in increasing the HDL and lowering the LDL component signifies a positive synergy between organic options and conventional management systems, as typified by the ability of *Lagenaria breviflora* in reversing the activities and mechanism of enzymatic and non-enzymatic antioxidants evidenced by the lipid peroxidation of LDL. It also strengthens the fact that HDL is inversely proportional to circulating LDL (Prasad *et al*., 2009) in the blood and also that the total number of eggs produced increases with reduction in serum LDL in laying birds (Peebles *et al* (2004).

The percentage of reduction (percent change) of yolk TC, LDL, VLDL and increase in HDL components was favoured in eggs sampled from the 200 g/4 litres dosed group compared to the other groups. This clearly shows a possible ability in influencing the quantity of good *Lipoprotein*s (increase) and undesirable *Lipoprotein* (reduction) in the overall content of the yolk. In addition, its (200 g/4 litres) combination with DL management system resulted in reduction of TC and LDL and improved HDL content to yolk deposition

The HDL-*Cholesterol* content of yolk was greatly improved in eggs sampled eggs from birds raised on DL and administered 200 g/4 litres LB while also exhibiting LDL lowering ability. This favours the use of LB in a conventional deep litter system and likewise on modified systems like DLR. In conclusion, commercial layers can be raised on the DL system and administered up to 200 g/4 litres of *Lagenaria breviflora* as a *Cholesterol* lowering agent in eggs without health impairment, thus favouring organic egg production.

Author's contribution

This work was carried out under the valuable advice and guidance of Ekunseitan D.A. and Adeyemi M.A while Akiode, Ikotun and Otubu (undergraduate students) carried out the field-work. Data compilation, statistical analysis and manuscript revision was done by Ekunseitan D.A. All authors read and approved the final manuscript.

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MORPHOMÉTRIE ET PRÉDICTION DU POIDS DU PORC LOCAL DANS DEUX RÉGIONS AU TOGO

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Résumé

L'élevage du porc local au Togo est dominé par le système extensif de type traditionnel avec un minimum d'intrants. Une enquête au sein de 497 élevages dans les régions Maritime et des Savanes a montré que cette activité connait une grande variabilité selon les exploitations et les types d'animaux élevés. Les analyses des données morpho biométriques collectées, sur 250 animaux issus de 75 exploitations sélectionnées suivant dix critères, ont permis d'établir que ces porcs ont i) un petit format, ii) une robe blanche (38%), noire (29,6%) ou blanc/noir (28,4%), et iii) des oreilles dressées (98,4 %). Les poids vifs moyens varient de 4,02 ± 2,49 kg à 2 mois ; 8,52 ± 5,67 kg entre 3 et 6 mois ; 15,80 ± 8,04 kg entre 7 et 12 mois à 23,76 ± 11,3 kg à plus de 12 mois. Les mesures de longueur du dos, de hauteur au garrot, du tour de poitrine, de longueur de la tête, de longueur des oreilles, de largeur des oreilles, de distance entre oreilles et de hauteur des onglons des pattes antérieures en plus de l'âge estimé ont servi à établir des équations de prédiction du poids. Il a été établi 129 équations de détermination du poids avec toutes ces dimensions. Le tour de poitrine prédit mieux le poids avec un coefficient de détermination R2 de 0,82 à 0,91 pour les quatre classes d'âge. Le modèle retenu est P = (5,6TP+0,8LD+0,3HG+Age–4LT–166,4) x 1/10 avec R2=0,89. En conclusion, cette méthode peut être utilisée pour déterminer le poids des porcs en milieu rural dépourvu de bascule.

Mots clés : porc local, barymétrie, poids vif, Togo.

MORPHOMETRY AND PREDICTION OF LOCAL PIG WEIGHTS IN TWO REGIONS OF TOGO

Abstract

Local pig farming in Togo is dominated by the extensive traditional system with a minimum of inputs. A survey carried out on 497 farms in the Maritime and Savanes regions showed that this activity is highly variable. It depends on farms and types of animals raised. Morphobiometric data were collected from 250 animals from 75 farms. These farms were selected according to ten criteria. The analysis showed that these pigs have i) a small size, ii) a white (38%), black (29.6%) or white/black (28.4%) coat, and iii) erect ears (98.4%). The average live weights ranged from 4.02 ± 2.49 kg at 2 months; 8.52 ± 5.67 kg at 3 to 6 months; 15.80 ± 8.04 kg at 7 to 12 months to 23.76 ± 11.3 kg at over 12 months. Back length, height at withers, chest circumference, head length, ear length, ear width, distance between ears and height of forelegs hooves in addition to the estimated age were measured to establish weight prediction equations. All of these dimensions were used to establish 129 weight determination equations. The chest circumference better predicts the weights of local pigs with a coefficient of determination R2 from 0.82 to 0.91 for the four age groups. The model used is P = (5.6TP+0.8LD+0.3HG+Age-4LT-166.4) x 1/10 with R2=0.89. In conclusion, this method can be used to determine the weight of pigs in rural areas without a scale.

Keywords: local pig, barymetry, live weight, Togo

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Introduction

L'utilisation des mensurations de certaines parties du corps des animaux pour estimer le poids, était connue sous le terme de zoométrie et appliquée en élevage de porc (Delage *et al*., 1955). La barymétrie qui consiste à déterminer le poids vif des animaux par des mensurations corporelles nécessite un matériel léger, un minimum de personnel et une contention réduite (Domingo, 1976 ; Landais, 1983 ; Larrat *et al*., 1985). Elle a été utilisée chez plusieurs races *Bovine*s en Afrique de l'Ouest (Dineur et Thys,1986 ; Fall *et al*., 1982, Planchenault, 1987). Cette connaissance du poids vif devient indispensable devant les techniques modernes d'élevages et de commerce du porc (Delate et Babu, 1990). Les moyens de pesée sont rares en milieu rural où sont élevés et vendus ces porcs. Il s'avère alors nécessaire d'appliquer cette technique d'estimation du poids sur le porc local au Togo. Il existe une diversité d'individus de porcs d'origines différentes sous l'effet des importations et des croisements anarchiques. L'objectif de cette étude est de caractériser ce porc et d'élaborer un modèle d'estimation de son poids le plus précis possible et utilisable en milieu rural.

Matériel et méthodes

Matériel

Site de l'étude

L'étude a été réalisée dans la Région Maritime (14% de l'effectif porcin national) et dans la Région des Savanes (34% de l'effectif porcin) (DSID, 2014). Au Togo, la végétation est caractérisée par un paysage de forêt dans la Région Maritime et un paysage de savane dans la Région des Savanes. La Région Maritime a un climat subéquatorial à deux saisons de pluies (mars à juillet et septembre à novembre). Elle couvre 6 100 km2 avec une population d'environ 2 600 000 hbts et compte huit préfectures. La Région des Savanes jouit d'un climat soudanien tropical avec une saison des pluies (mai à octobre) et une saison sèche (novembre à avril). Elle a une superficie de 8 600 Km2 répartie en cinq préfectures et compte environ 660 000 hbts (DGSCN, 2010). L'étude est menée dans toutes les préfectures des deux régions. (Figure 1)

Matériel de l'étude

Matériel animal

Il est constitué de porcs reconnu local au Togo. Dans chaque élevage, les animaux concernés sont constitués du couple (verrat, truie) et de deux de leur descendant de la dernière portée.

Matériel technique

L'enquête est réalisée à partir d'une fiche de collecte des mensurations des zones anatomiques précises des porcs.

L'échantillonnage a d'abord porté sur les élevages de porc local choisis à partir de la base de données des élevages de porcs (DSID, 2014). Les principaux critères obligatoires du choix des élevages sont :

- le nombre d'années d'expérience en élevage du porc local (au moins 5 ans) ;
- l'existence d'une porcherie (pour éviter la divagation) ;
- l'effectif des porcs (une dizaine de têtes au moins)
- et l'adhésion à l'objectif de l'étude (caractérisation).

Sur la base de ces critères, 75 élevages ont été retenus pour la collecte des données. La collecte des données est réalisée par la même personne dans tous les élevages.

Le matériel technique est constitué d'un mètre ruban flexible de 150 cm de long et de précision 1 mm pour les mensurations, et un peson à ressort (marque SALTER) de 100 kg de capacité et de précision 200 grammes pour les pesées. La contention est assurée à l'aide d'un sac de jute.

Collecte et traitement des données

La collecte des données individuelles s'est basée sur les recommandations de la Fao (2012) dans ses directives et a retenue (08) huit zones anatomiques pour les mensurations.

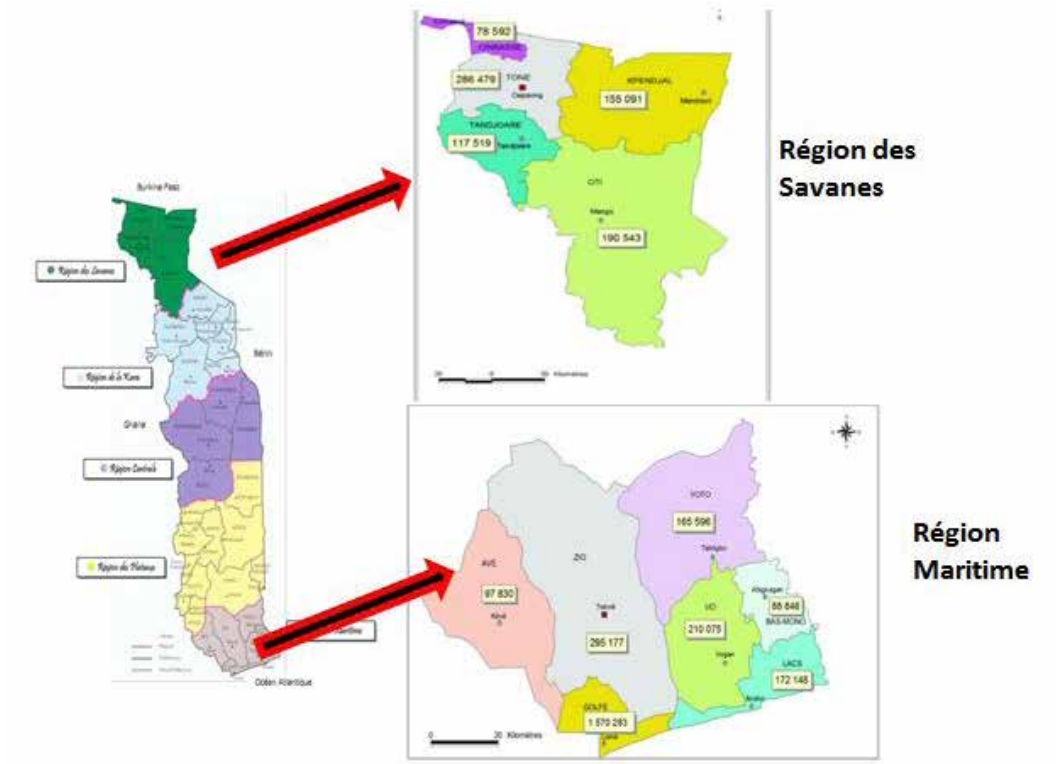

Figure 1 : Carte du Togo et des deux régions de l'étude

Il s'agit de la longueur du dos (LD), la hauteur au garrot (HG), le tour de poitrine (TP), la longueur de la tête (LT), la longueur des oreilles (LoO), la largeur des oreilles (LaO), la distance entre oreilles (DO) et la hauteur des onglons des pattes antérieures (HoPa). Les mesures sont faites sur les porcs immobilisés sur pieds et les pesées effectuées après mensurations. Les données ainsi collectées sont traitées à l'aide du logiciel SPSS 20 IBM. Les animaux sont regroupés en quatre classes d'âge qui sont :

- avant sevrage (0 à 2 mois),
- phase de croissance (3 à 6 mois),
- stade d'exploitation (7 à 12 mois)
- les porcs de plus d'un an.

Les équations de régression linéaires simples et multiples sont élaborées pour chaque classe d'âge. Le poids à âge type est la variable expliquée et les différentes mesures constituent les variables explicatives. Le modèle s'écrit sous la forme : $P= \alpha 0+ \alpha 1M1+ \alpha 2M2+\ldots. +\alpha nMn$. Les distances de Mahalanobis et de Cook sont calculées pour la sélection des meilleurs modèles.

Résultats

La collecte des données est réalisée dans 75 élevages dont 68% dans la Région Maritime et 32% dans la Région les Savanes. Cette collecte a porté sur 250 sujets dont 158 dans la Maritime et 92 dans celle des Savanes. L'âge des animaux varie de deux jours à 72 mois. Répartis en classe d'âge, on a trouvé 10,4% de porcelets avant sevrage, 22,4% en phase de croissance contre 33,6% au stade d'exploitation et 33,6 pour les porcs de plus d'un an.

Caractéristiques générales des animaux

Format : le porc local a une longueur qui varie de 18 à 123 cm avec une taille de 15 à 78 cm de haut. La tête se présente sous une forme cylindro-conique avec une longueur variant entre 6 cm (à la mise bas) à 46 cm à l'âge adulte. Les oreilles sont plus longues (3 à 22 cm) que large (3 à 16 cm) et distantes de12 cm au plus. La majorité des animaux (98,4%) ont les oreilles dressées. De façon générale il est observé un

faible poids des animaux car le poids a varié de 0,5 kg à la naissance à 22,6±9,76 pour les truies et 28 28±15,32 pour les verrats. (Tableau 1).

La robe : Différentes couleurs de robe sont retrouvées dont : le blanc (38%) ; le noir (29,6%) ; la pie noire / noire pie (27,6%) ; le gris (2,4%) et le fauve (0,8%).

Barymétrie

A l'analyse, une prédiction de poids calculé par variable est déterminée sous la forme P=αe^βx. Pour chaque variable, un nuage de points a présenté la position des poids des 250 animaux considérés (Figure 2).

En considérant toutes les variables, 129 équations ont été établies pour chaque classe d'âge afin d'estimer le poids à divers degrés de précision. Le coefficient de détermination R^2 relatif à chaque équation a permis de dégager un modèle par séries de variables (Tableau 2)

Les équations retenues pour la prédiction du poids des porcs

Pour toutes les classes d'âges regroupées, l'équation qui explique le mieux est celle dont le coefficient de détermination est de 0,895 et qui prend en compte toutes les neuf variables. (Tableau 3). La détermination du poids en fonction de l'âge est estimée selon les coefficients de corrélation. Le tableau 4 permet de déterminer les variables qui prédisent mieux le poids à âge type (PAT) en fonction des coefficients de corrélation. D'où l'établissement de l'équation P= (5,6TP + 0,8LD + 0,3HG + Age – 4LT – 166,4)1/10. L'équation donne le poids (P) en kg, à partir des mensurations en cm et l'âge en mois.

Tableau I : Variation du poids et des mensurations en fonction des classes d'âge.

Paramètre		**1er âge**	**2ème âge**	**3ème âge**	**4ème âge**	**Moyenne globale**
Poids (kg)	Min – Max	0,5 – 9	2 – 31	4,5 – 38	7 – 73	0,5 – 73
	Moy ± ET	4,02±2,49	8,52±5,67	15,80±8,04	23,76±11,29	15,61±10,93
LD (cm)	Min – Max	18 – 50	28 – 79	39 – 89	35 – 123	18 – 123
	Moy ± ET	35,15±9,14	48,39±11,17	61,49±12,61	70,83±12,28	58,96±16,54
HG (cm)	Min – Max	15 – 40	21 – 56	30 – 60	33 – 78	15 – 78
	Moy ± ET	26,87±6,78	34,84±7,05	43,04±8,64	49,8±7,13	41,77±10,66
TP (cm)	Min – Max	19 – 54	15 – 82	38 – 91	44 – 141	15 – 141
	Moy ± ET	35,9±9,41	47,48±11,81	61,99±12,8	72,88±13,84	59,69±17,64
LT (cm)	Min – Max	10 – 21	6 – 27	16 – 31	21 – 46	6 – 46
	Moy ± ET	15,04±3,29	19,38±3,80	23,92±3,29	27,18±3,42	23,07±5,25
LoO (cm)	Min – Max	3 – 9	4,5 – 14	6 – 12	6 – 22	3 – 22
	Moy ± ET	5,6±1,6	7,29±1,95	8,51±1,47	9,79±2,28	8,36±2,3
LaO (cm)	Min– Max	3 – 9	4 – 12	5 – 11	5 – 16	3 – 16
	Moy ± ET	5,31±1,55	6,75±1,59	5,97±1,39	8,71±1,79	7,69±1,72
DO (cm)	Min – Max	3 – 7	4 – 9	5 – 10	5 – 12	3 – 12
	Moy ± ET	5,21±0,93	6,46±1,14	7,51±1,15	8,52±1,36	7,38±1,6
HoPa (cm)	Min – Max	1 – 2	1 – 3	1,5 – 3,5	1,5 – 4	1 – 7
	Moy ± ET	1,33±0,4	1,93±0,49	2,34±0,47	2,67±0,51	2,27±0,69
Effectif n (%)		26 (10,4)	56 (22,4)	84 (33,6)	84 (33,6)	250

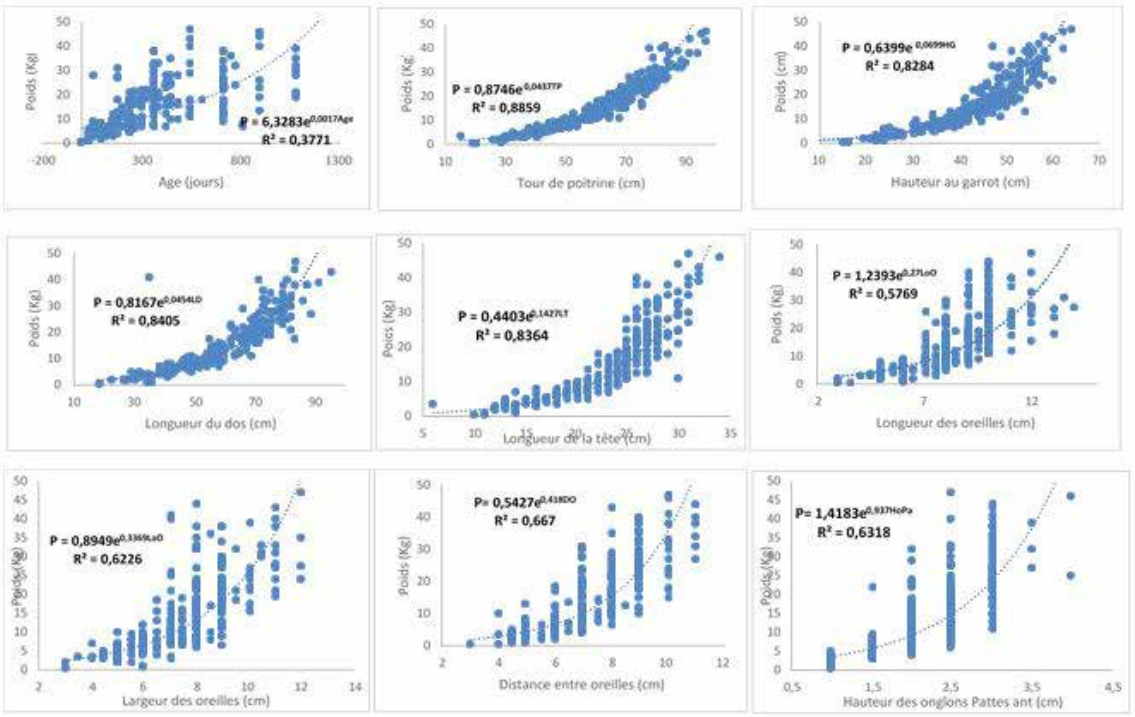

Figure 2 : Courbes des différentes variables en rapport avec le poids

Tableau 2 : Liste des équations retenues pour tous les âges

Nombre de variables	Nombre d'équations possible	Equation de prédiction du poids	R^2
Une	9	P= 0,58TP – 19,18	0,885
Deux	36	P= 0,54TP + 0,59DO – 20,98	0,888
Trois	28	P= 0,53TP + 0,03LD + 0,08Age -18,78	0,888
Quatre	21	P= 0,54TP + 0,003LD + 0,014HG + 0,59DO – 21,07	0,888
Cinq	15	P= 0,57TP + 0,08LD + 0,03HG – 0,37LT + 0,11Age – 16,64	0,891
Six	10	P= 0,55TP + 0,06LD + 0,04HG – 0,43LT + 0,58DO + 0,09Age – 18,14	0,893
Sept	6	P= 0,55TP + 0,05LD + 0,03HG – 0,46LT + 0,26LoO + 0,54DO + 0,1Age – 18,15	0,894
Huit	3	P= 0,55TP + 0,05LD + 0,03HG – 0,46LT + 0,25LoO + 0,03LaO + 0,54DO + 0,1Age – 18,17	0,894
Neuf	1	P= 0,55TP + 0,05LD + 0,02HG – 0,52LT + 0,21LoO + 0,08LaO + 0,52DO + 0,73HoPa + 0,1Age – 17,91	0,895

Discussion

Au Togo, on ne dispose pas d'un référentiel de porc local alors il est difficile de le reconnaître sur le terrain et sur le plan génétique. Or, le porc local est largement élevé en milieu rural avec différents croisements aux races importées. Ceci explique cette étude qui permet de décrire d'une part ce porc et d'autre part d'estimer son poids à partir des variables anatomiques. Les élevages sont choisis sur la base des données disponibles chez les techniciens de terrain (Somenutse *et al*., 2018 sous presse). Les différentes robes rencontrées sur le terrain sont le fruit des multiples croisements dans le but d'amélioration de la race. Les résultats montrent que le système d'élevage influence le poids final qui se situe à 23,76±11,29. Les mensurations réalisées sur les zones anatomiques définies permettent une estimation du poids de ces animaux. L'élevage porcin traditionnel se caractérise par une minimisation des intrants et des investissements. En plus, l'alimentation et la conduite demeurent hasardeuses. Ceci peut influencer le poids final des porcs adultes comme le stipulent certains auteurs en signifiant que c'est le bien être qui peut permettre à l'animal d'exprimer toutes ses aptitudes (Dourmad, 2010 ; Dourmad *et al*., 2010 ; Paboeuf *et al*., 2010). En milieu rural, la vente du porc se fait sur une estimation visuelle du poids car l'emploi de bascules pèse-bétail nécessite un véhicule, du personnel et certains aménagements. Cette pratique s'avère impossible et coûteuse en raison de la situation des voies de communication et des moyens matériels disponibles (Landais, 1983) d'où l'alternative à utiliser la barymétrie. Pour Voisin *et al*. (2011), la prédiction du poids d'un porc en utilisant la simple mesure de son TP est plus précise que l'évaluation visuelle. Elle est également plus rapide que la mesure de plusieurs repères anatomiques. Toutefois, dans le cadre de la présente étude, la méthode des recommandations de la Fao (2012) est choisie pour donner plus de fiabilité aux informations recueillies. A l'issue des analyses, il est ressorti de notre étude que les quatre mensurations anatomiques (TP, R2=0,88 ; LD R2=0,84 ; LT R2= 0,83 ; HG R2=0,83) et l'âge (R2=0,4) sont les variables les plus prédictives avec R2=0,9. Des équations prenant en compte deux ou trois mensurations les plus étroitement liées au poids vif, permettraient de diminuer sensiblement les marges d'erreurs. Dans leurs travaux sur les porcs créoles, Delate et Babu (1990) ont utilisé 14 mensurations mais confirment également que c'est le tour de poitrine qui explique mieux le poids des porcs. Quels que soient les types génétiques, le tour de poitrine donne la plus forte corrélation avec le poids suivi par la longueur du tronc et la hauteur au garrot (Faarungsang et Chantsawang, 1982 ; Sahaayaruban *et al*., 1984 ; Tahman *et al*., 1983). Chez les autres espèces animales, les mêmes variables anatomiques sont exploitées et les auteurs retrouvent que le tour de poitrine donne les meilleurs résultats notamment dans les travaux de Dodo *et al*., (2001). Ainsi, les résultats obtenus avec les modèles ne sont pas significativement différents des poids réels ce qui prouve la précision dans la construction des modèles de régression. Il ressort que les variables ne sont pas les mêmes suivant les classes d'âge ; ceci peut s'expliquer par la variabilité des souches génétiques des individus de l'étude.

Conclusion

Cette étude établit une description phénotypique du porc local. La méthode utilisée a permis d'aboutir à des résultats sommaires. Toutefois, ces résultats sont nécessaires pour un travail plus poussé qui utilisera les marqueurs microsatellites en vue de permettre une meilleure caractérisation génétique de ces porcs. La prédiction des poids à partir des mensurations anatomiques a permis l'établissement d'une équation retenue qui peut être d'une utilisation courante sur le terrain en absence de moyens de pesée.

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

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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.

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 *general*ly 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 *generat*ed 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 weeks 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).

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
Bone meal	1.50	1.50	1.50	1.50	1.50
Limestone	0.10	0.10	0.10	0.10	0.10
Premix	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Toxail binder	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

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.

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

		SRC		RRC			
Parameter	**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

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

		SRC		RRC			
Parameter	**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.

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

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

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

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 the 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 significanct differences in the weights of spleens between the treatment groups. The results of 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*

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

		SRC		RRC		
Parameter	**A (0%)**	**B (5%)**	**C (10%)**	**D (5%)**	**E (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^{NS*}
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

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 diets 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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EFFECT OF LENGTH AND STORAGE METHODS ON THE CHEMICAL COMPOSITION OF EXOTIC CHICKEN AND QUAIL EGGS

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Abstract

The quality characteristics and proximate composition of the eggs of chicken and quail were compared. A total of 200 chicken eggs were collected from 28 weeks old Harco layers and 200 Japanese quail (Coturnix coturnix japonica) eggs from 21 weeks old quail birds. Eggs were randomly selected and divided into 5 groups of 10 eggs per group for both chicken and quail eggs respectively and stored for 21 days. The eggs were subjected to different storage methods (room temperature, refrigeration, oiling, black polythene bags) with room temperature as control and observed at different intervals 0, 3, 7, 14, 21days.

Weight loss was measured and proximate analysis was carried out. Data was analysed using analysis of variance (ANOVA) with treatments (storage methods) and durations as the 2 main effects. For all treatments, the values related to egg weight decreased as weight loss increased from day 0 to 21. The proximate composition for all storage methods were not significantly different ($P > 0.05$) from each other throughout the duration of storage.

Comparison of the length and method of storage of both chicken and quail eggs shows that they can both be stored for 3 days without deterioration using any of the four storage methods. In both eggs, refrigeration was the best storage method even though the use of polythene bags could be an alternative for chicken eggs while oiling could be an alternative for quail eggs when stored for longer periods.

Key words: Proximate composition, egg weight loss, egg quality, storage methods, length.

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Introduction

Egg is one of the richest and most balanced sources of nutrients among all of the foods available to mankind. It has become extremely important throughout the world nutritionally as well as economically (Ricketts, 1981; Scott & Silverside, 2000). It is a cheap source of protein and provides a balanced protein which contains all the amino acids considered essential in sufficient amounts and proportion to maintain life and support growth when used as a sole source of protein food (Ricketts, 1981; Adeogun and Amole, 2004). It contains an abundant supply of minerals that are essential for building and also for maintaining a healthy body.

Eggs are *general*ly similar among species of birds, but they can differ in some aspect in their physical and chemical composition (Richard, 2008). The exotic chicken eggs (*Gallus domesticus*) are the most common known type of avian eggs among consumed but the Japanese quail (Coturnix coturnix japonica) also has a high rate of egg production and its eggs are also nutritious (Adeogun and Amole, 2004).

Figure: Chicken and quail eggs

Foods are known to have shelf lives which vary depending on the type of food. Egg is also a very perishable food product with a short shelf life which could lose its quality rapidly during the period between its storage and consumption. The freshness of an egg is very important to the consumer as it is a factor of how recently an egg was laid; other factors could be the temperature at which it is held, humidity and the handling. These variables are so important that an egg at one week old, held under ideal storage conditions (temperature around 40c and relative humidity of between 70-80 %) can be fresher than an egg left at room temperature for one day (Keener *et al*., 2005). The internal quality of eggs starts to decline as soon as they are laid by hens. Poor storage conditions may result in the deterioration of egg quality leading to the loss and wastage of eggs which has been a major concern for poultry farmers (Jin et. al. 2011). Studies have shown that long storage periods are detrimental to table and hatching egg qualities (Samli et. al., 2005; Scott et. al., 2000; Walsh *et al*., 1995).

Egg quality comprises a number of aspects related to the shell, *Albumin* and yolk and may be divided into external and internal quality. The quality of an egg is based on the following: cleanliness, freshness, egg weight, shell quality; yolk index, albumen index, Haugh unit and its chemical composition (Kul and Seker, 2004). The nutritional and functional properties of an egg can be affected by storage time, method of storage, temperature and humidity of storage. Storage can modify some characteristics of the egg including loss of water, carbon dioxide and a subsequent increase on the pH of the albumen (Decuypere et. al. 2001)

A number of studies have been conducted concerning the effects of storage length and methods of storage on egg quality. However, the effects of these methods and length of storage on the chemical composition of chicken and quail eggs are not fully known. Therefore, the objective of this study was to examine and also to compare the effects of the length and methods of storage on the chemical composition of Japanese quail and exotic chicken eggs.

Materials and Methods

A total of 200 chicken eggs (*Gallus domesticus*) of almost equal size collected from 28 weeks old black harco layers in the

poultry unit of the Teaching and Research Farm of Obafemi Awolowo University, Ile- Ife, and 200 Japanese quail eggs of almost equal size collected from 21 week-old Japanese quails (Coturnix coturnix japonica) from the National Veterinary Research Institute (NVRI), Ikire, Osun State was used. The eggs collected were used within 24hrs of lay.

Fifty eggs were randomly selected from the collection for chicken and Quail eggs respectively and were divided into 5 groups of 10 eggs per group, treated and stored for 21 days for each of the following methods:

- *Room temperature (control):* Eggs were placed in egg trays untreated and stored at room temperature (30°c).
- *Black polythene bag:* Eggs were stored in a black polythene bag with the mouth tied and stored at room temperature (30°c).
- *Oiling:* Eggs were immersed in vegetable oil, allowed to drain for some seconds and stored at room temperature (30°c).
- *Refrigeration:* Eggs were arranged on egg trays and stored in a refrigerator at 18°c.

Eggs were measured at each observation period (0, 3, 7, 14, 21 days). The eggs were marked to avoid mix-up. The measurements taken at day 0 were used as the control for the length of storage for each storage method. The external quality parameters were measured:

- Egg weight (g) was obtained at day 0 for all eggs and at each day of observation for each treatment using the sensitive scale balance.
- Egg width was measured for each egg at each day of observation using a vernier calliper.
- Egg weight loss was determined as the difference between successive weights of eggs on different weighing days.

For determination of chemical composition, eggs were broken for each treatment on each day of observation (0, 3, 7, 14, and 21) and the liquid egg for each treatment was poured into a separately labelled nylon container for identification of each sample. Proximate analysis was then carried out on the samples at the meat laboratory of the department of Animal sciences (OAU) as described by AOAC (1990).

Moisture content: 2g of each sample from each treatment and length of storage was weighed into a crucible (platinum, ceramic) using the mettler analytical balance to determine its initial weight. It was then oven-dried for 24 hours at a temperature of 70°c. The final weight of the sample was estimated by using the mettler analytical balance with the following expression:

Moisture content (%):

$$\frac{\textit{Initial weight (g) - Final weight (g)}}{\textit{Weight of sample taken (g)}} \times 100$$

- *Ash content:* The residual sample from the estimated moisture content was used to determine the ash content. This was estimated by burning off completely all the organic constituents in a furnace at 600^0c for 3 hours and allowing it to cool before taking the final weight of the sample using the mettler analytical balance. The percent ash content was estimated by using this expression:

$$\frac{\textit{[Weight of empty crucible+ ash sample] – Weight of empty crucible}}{\textit{Weight of sample taken (g)}} \times 100\%$$

- *Ether extract:* An empty beaker was weighed and 3g of the sample was weighed in another beaker. The weighed sample was transferred into a separating funnel and 20ml of N-hexane was added and mixture was shaken for 15 minutes. The ethered layer was collected into the empty beaker and was dried for 24 hours at 70°c. The final weight was then taken using the analytical balance.

$$\frac{\textit{[Weight of empty beaker+oil] – Weight of empty beaker}}{\textit{Weight of the sample (g)}} \times 100\%$$

- *Crude protein:* 0.5g of the sample was weighed into a kjeldahl flask and 20ml of concentrated sulphuric acid (H_2so4) was

added and also a little scoop of digestion catalyst. The sample was digested using the kjeldahl digestion system for 2 hours. The digested sample was then left to cool and collected into a labelled sample bottle and made up to 50ml with distilled water, this was followed by distillation of the ammonia in the sample. A final process of back titration using 0.0105 ml concentrated hydrochloric acid and 2% of boric acid and indicator was used so as to get a sharp colour of boric acid at the end point. Percent crude protein was estimated as follows:

% Total Nitrogen (N) =

$$\frac{\textit{Titre value} \times \textit{Acid conc.} \times \textit{Molar mass of N} \times \textit{dilution factor}}{\textit{Weight of Sample}} \times 100$$

% Crude protein = % Total Nitrogen × 6.25

Data collected were subjected to analysis of variance to determine significant differences (P< 0.05) using the Statistical Analysis System (SAS, 2003) with the treatments (Storage methods) and duration of storage as the two main effects. Means were subsequently separated using Duncan's Multiple Range test.

Results and Discussions

The effect of duration on egg quality parameters of chicken and quail eggs are shown in Table1. Egg weight loss for chicken and quail progressively increased for all the storage durations. There was significant difference (P<0.05) in the moisture content of chicken eggs at the different storage durations. The moisture content of chicken eggs at day 0 was significantly (P<0.05) lower than that of the other storage durations. The moisture contents for day 7 to day 21 were not significantly different from each other. There was no significant (P>0.05) difference in the moisture content of quail eggs at the different storage durations. An increase in the duration of storage results in increase in weight loss of chicken and quail eggs.

There was no significant difference (P>0.05) in the ash content of chicken eggs at the different storage durations. The ash content of quail eggs stored for 3 and 7 days were significantly (P<0.05) lower than that of the other storage durations. There was no significant difference (P>0.05) in the ether extract content of the quail eggs stored for different days while the chicken eggs stored for 14 days was significantly (P<0.05) higher than that of 3 and 7 days duration of storage.

Table 1: Effect of Duration on Egg Quality Parameters of Chicken and Quail eggs

		Duration of Storage					
Parameters	**Egg type**	**0**	**3**	**7**	**14**	**21**	**SEM**
WL	Chicken	-	0.17^{b}	0.54^{b}	1.23^{ab}	1.97^{a}	0.30
	Quail	-	0.74^{b}	1.30^{b}	1.83^{ab}	3.01^{a}	0.33
MC	Chicken	73.71^{c}	77.74^{a}	76.32^{b}	76.21^{b}	76.21^{b}	0.17
	Quail	70.90	73.82	73.65	74.30	69.72	1.05
Ash	Chicken	0.82	0.72	0.86	0.85	0.81	0.04
	Quail	1.07^{a}	0.97^{b}	0.82^{b}	1.12^{a}	1.15^{a}	0.06
EE	Chicken	1.71^{ab}	0.73^{c}	1.53^{bc}	2.59^{a}	1.94^{ab}	0.23
	Quail	0.97	1.09	1.92	1.46	1.35	0.20
CP	Chicken	8.53^{b}	9.19^{ab}	11.14^{a}	11.61^{a}	10.69^{ab}	0.66
	Quail	10.71	10.49	10.72	11.12	12.66	0.52

^{a,b,c}: Means within each row with different superscripts are significantly different (P<0.05)
Key: SEM = Standard Error of Mean, WL = Weight loss, MC = Moisture content, Ash = Ash content, EE = Ether extract content, CP = Crude protein content

The crude protein content of the chicken eggs using all the storage methods across the days of storage was significantly different from each other. The crude protein of chicken eggs at day 0 was significantly ($P<0.05$) lower than those stored for 3 and 7 days while there was no significant difference ($P>0.05$) in crude protein of quail eggs at the different storage durations.

Effects of storage methods on the egg quality parameters of chicken and quail eggs are shown in Table 2. The results showed that the weight loss, moisture content, ash, ether extract and crude protein of chicken and quail eggs for all the storage methods were not significantly different from each other.

For both Chicken and quail eggs, weight losses increased with an increase in storage length. A high weight loss was observed for both types of eggs stored for 14 and 21 days. This could be due to loss of certain gaseous moleclules (CO_2, NH_3, N_2, H_2S) and water from the egg (Ihekoronye and Ngoddy, 1985; Decuypere *et al*. 2001; Akyurek & Okur, 2009). The reduced weight loss observed with oiling could be due to blockage of air-pores. The chemical composition of the two types of egg at day 0 differs from other days, this is in accordance with the work of Jin *et al*., (2011)

Table 2: Effect of Storage methods on Egg Quality Parameters of Chicken and Quail eggs

		Methods of storage					
Parameters	**Egg type**	**RT**	**Oil**	**PB**	**RF**	**SEM**	**P**
WL	Chicken	1.73	0.97	0.60	0.63	0.44	0.35
	Quail	2.41	1.37	1.12	1.99	0.52	0.38
MC	Chicken	75.78	75.86	76.12	76.08	0.63	0.98
	Quail	71.62	72.96	72.79	72.60	1.17	0.93
Ash	Chicken	0.86	0.80	0.83	0.75	0.04	0.27
	Quail	1.08	0.99	0.98	1.06	0.08	0.79
EE	Chicken	1.24	1.84	1.98	1.72	0.34	0.54
	Quail	1.30	1.42	1.11	1.59	0.23	0.56
CP	Chicken	10.02	10.04	10.34	10.82	0.81	0.90
	Quail	11.33	11.60	10.43	11.18	0.57	0.60

[a, b, c]: Means within each row with different superscripts are significantly different ($P<0.05$)
Key: RT = Room temperature, Oil = Oiling, PB = Polythene bag, RF = Refrigeration
SEM = Standard Error of Mean, WL = Weight loss, MC = Moisture content, Ash = Ash content,
EE = Ether extract content, CP = Crude protein content

Table 3: Effect of the studied factors and their interactions in the factor model analysis of variance

Source of variation	**WL**	**MC**	**Ash**	**EE**	**CP**
	P > F	**P > F**	**P > F**	**P > F**	**P > F**
ET	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MS	0.0003	<0.0001	Ns	<0.0001	0.01
DS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ET*MS	0.001	<0.0001	Ns	<0.0001	<0.0001
ET*DS	ns	<0.0001	0.0004	<0.0001	<0.0001
MS*DS	0.01	<0.0001	0.01	<0.0001	<0.0001
ET*MS*DS	ns	<0.0001	0.002	<0.0001	<0.0001

Key: ET = Egg type, MS = Method of storage, DS = Duration of storage
SEM = Standard Error of Mean, WL = Weight loss, MC = Moisture content, Ash = Ash content,
EE = Ether extract content, CP = Crude protein content, ns = not significant ($P>0.05$).

and Mohammed (2011) who reported that the internal quality of eggs starts to decline as soon as they are laid by hens.

The effects of the studied factors and their interactions in the factor model analysis of variance are shown on Table 3. No interactions were apparent ($P > 0.05$) among egg types and methods of storage for ash content, egg type and duration of storage, egg type × method of storage × duration of storage for weight loss. Strong interactions were found for the moisture content, ether extract content and crude protein content with P values of <0.0001.

Conclusion

From this study, it can be concluded that chicken and quail eggs can be stored for three days without effect on their chemical composition when any of the storage methods is used. Refrigeration could be used to properly store chicken and quail eggs for more than 3 days.

For chicken eggs, the other storage method that could also be good after refrigeration is the use of polythene bags while after refrigeration for quail eggs, oiling is also a good storage method.

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CLINICO-PATHOLOGICAL EFFECTS OF SINGLE AND MIXED *ESCHERICHIA COLI* AND *SALMONELLA GALLINARIUM* INFECTION IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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Abstract

The study was designed to determine the clinical outcomes in African Catfish experimentally infected with single and mixed *Escherichia coli* and *Salmonella gallinarum* strains. One hundred and sixty fish divided into four groups (A, B,C and D) of 40 per group were used for the study. Clinical isolates of *E.coli* and *S.gallinarum* were used to infect the fish by immersing them in water containing $1x10^8$ colony forming units/ml of each isolate. Group A, B and C were infected with *E.coli*, *S.gallinarum* and mixed *E.coli* and *S.gallinarum* respectively while the fish in group D served as uninfected controls. Fish in the infected and control groups were monitored daily for five weeks post infection for signs of ill-health and mortality. Sluggish movement, cuddling together, emaciation, poor growth rate, anorexia, high morbidity and mortality were the signs of ill health observed in fish in the infected groups. The clinical signs were more pronounced in the groups A and C when compared to group B. The uninfected control showed no obvious clinical manifestations. The weight decreasing effect of the infection was significant ($p<0.05$) in groups A and C, being more in A than C. The weight of group B was comparable to group D. Grossly, enlarged livers and multiple areas of focal necrosis were observed in group A. Histopathology results revealed multiple golden yellow depositions (*haemosiderosis*) on the spleen of fish in group B. This study has shown *E. coli* and *S. gallinarum* can adversely affect the health and productivity of cultured African catfish.

Key words: *Escherichia coli*, *Salmonella gallinarum*, *Clarias gariepinus*

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Introduction

African Catfish (*C. gariepinus*) is one of the most important individual species in traditional freshwater fisheries in Africa (Skelton, 2001). It is widely distributed in Africa, where it occurs in almost any fresh water habitat, but favours floodplains, large sluggish rivers, lakes and dams (Skelton, 2001). *Clarias* species are a highly esteemed group of fish in Nigeria and have a very high commercial value in the market (Huisman and Fitcher, 1987). Intensive production of fish increases the likelihood of and severity of parasite and disease outbreaks which constitute a major constraint to aquaculture (Ilhan and Ilknur, 2003). Bacterial infections are among the most common and troublesome diseases of fish reared in ponds and are responsible for heavy mortality in both wild and cultured fish and a cause of substantial financial loss in aquaculture production (Bandart, 2000).

The colonization of fishes by various parasites from *Faecal* sources of pollution has impacted on diseases such as salmonellosis (Williams *et al*., 1989). Guzman *et al* (2004) reported that invasion of fish muscle due to the breakage of the immunological barrier of fish by pathogens is likely to occur when the fish are raised in ponds with *Faecal* coliforms (*E. coli* and *S. gallinarum*) of greater than 10^3 /ml in pond water. The present study seeks to document the clinical and pathological manifestations of *S. gallinarum* and *E. coli* infection (either as a single or mixed infection) in *C. gariepinus*.

Material and Methods

Experimental animals: One hundred and sixty apparently healthy 6-weeks-old *Clarias gariepinus* post fingerlings of mean weight of 33.63 g were used. They were acclimatized for 2 weeks during which period 2 mm fish basal diet was provided twice daily (Sarka and Rahid, 2012). The culture water was changed once in 2 days by the gradual removal and addition of fresh water to ensure adequate oxygenation. The fish were randomly assigned into four groups (A, B, C and D) of 40 catfish per group. Each group was further subdivided into groups of 10 fish per sub group for ease of handling and reared in transparent plastic tanks measuring 46 cm by 29 cm by 23 cm with a capacity of 30.7 L each.

Infection of the fish: *Escherichia coli* and *Salmonella gallinarum* strains were obtained from the department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. Three to four colonies of *Escherichia coli* and *Salmonella gallinarum* strains were homogenized separately in sterile phosphate buffered saline and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard (equivalent to $1x10^8$ colony forming units/ml). Fish in groups A, B and C were infected by immersing them in appropriate tanks containing 1ml/litre of bacterial inoculum as follows: group A (*Escherichia coli* only), group B (*Salmonella gallinarum* only), group C (*E. coli* and *S. gallinarum*) and group D served as the uninfected control. Infected and uninfected fish were observed for 5 weeks.

The infected fish were monitored daily for signs of ill health: sluggishness, off feed, morbidity, mortality and skin lesions. Infection was confirmed by re –isolating the bacteria from the skin and gastro-intestinal tracts of dead and/ or moribund fish from the different groups randomly selected at the end of the experimental period of 5 weeks. Eosine-methylene blue (EMB) agar and Salmonella-Shigella agar were used for the isolation of *E. coli* and *S. gallinarum* respectively. The absolute weight of the fish in the different groups was obtained weekly throughout the period of the experiment. A total of twenty fish from different groups, five from each of the subgroups were randomly selected and their individual body weights were determined using an electronic sensitive weighing balance. The mean weight for each group was obtained by summing the individual weights for the subgroups and divided by 5. Their values were obtained and recorded.

Gross pathology: Three fish from each sub-group were randomly selected and sacrificed by blow to the head at 5 weeks post infection. Following death, the fishes were

dissected and the internal organs examined for presence of gross lesions.

Histopathology studies: Specimens of liver, spleen and intestine were cut and fixed in bouin's fluid for 48hrs. Later these specimens were dehydrated in increasing concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Five micro meter (5µm) thick sections were cut, mounted on glass slides and stained with haematoxylin and eosine (H&E) for light microscopy. Photomicrographs were captured using a motican camera.

Data presentation and analysis: Clinical outcome, morbidity and mortality rates were determined and presented in tables, while data on absolute body weights among the four experimental groups were compared using a one way analysis of variance (ANOVA). Significant difference was accepted at ($p < 0.05$).

Results:

Clinical signs

The onset of clinical signs was observed at four days post infection and lasted until five weeks post infection. There was no morbidity in groups B and D but groups A and C showed 100% and 50% morbidity respectively. Fish in group A and C showed sluggish movement, cuddling together and poor growth rate which was absent in groups B and D as presented in Table 1.

Anorexia was observed in group A where the fish were completely off feed while group C had depressed appetite but groups B and D were feeding adequately. There was no morbidity in groups B and D but groups A and C showed 100% and 50% morbidity respectively. Fish in groups A and C were emaciated when compared to groups B and D as shown in plate

Table 1: Clinical signs observed in *Clarias gariepinus* five weeks post infection with single and mixed *E.coli* and *S.gallinarium*

	Experimental Group			
Clinical parameter	**A (*E.coli*)**	**B (*S.galli*)**	**C (*E.coli* + *S.galli*)**	**D (Uninfected Control)**
Sluggish Movement	Present	Absent	Present	Absent
Anorexia	Completely off feed	Absent	Depressed appetite	Absent
Morbidity rate	100%	Absent	50%	Absent
Cuddling together	Present	Absent	Present	Absent
Emaciation	Severely emaciated	Absent	Slightly	
Emaciated	Absent			
Growth rate	Poor	Good	Poor	Good
Mortality rate	0%	0%	5%	0%

Table 2: Mean weight post infection (grams) of *Clarias gariepinus* infected with single and mixed *E.coli* and *S. gallinarium*.

	Experimental Group			
WEEK post infection	**A**	**B**	**C**	**D**
0	$32.08^{a} \pm 2.46$	$34.00^{a} \pm 3.61$	$34.18^{a} \pm 1.75$	$34.35^{a} \pm 0.98$
1	$51.25^{b} \pm 4.25$	$67.75^{a} \pm 4.22$	$63.43^{a} \pm 4.63$	$74.95^{a} \pm 1.43$
2	$49.90^{c} \pm 3.62$	$62.65^{ab} \pm 3.83$	$58.25^{bc} \pm 4.11$	$71.50^{a} \pm 3.62$
3	$54.13^{c} \pm 4.24$	$67.40^{ab} \pm 3.56$	$63.35^{bc} \pm 4.30$	$77.43^{a} \pm 2.93$
4	$60.58^{b} \pm 4.81$	$72.40^{ab} \pm 5.40$	$71.40^{ab} \pm 6.52$	$81.68^{a} \pm 3.23$
5	$43.50^{c} \pm 2.06$	$89.75^{ab} \pm 3.25$	$61.25^{bc} \pm 7.69$	$95.25^{a} \pm 17.50$

Mean weights with the same superscript on the same row are not significantly different ($p > 0.05$) while those of different superscripts are significantly different ($p \leq 0.05$)

Figure 1: Fish from group A showing Emaciation (Big head, long body and tail) at 5 weeks post infection.

Figure 2: Area of focal necrosis on the liver of a fish in group B at 2 weeks post infection

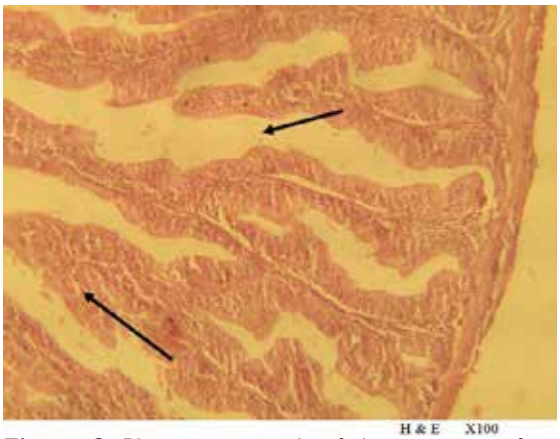

Figure 3: Photomicrograph of the intestine of an African Catfish experimentally infected with *E. coli* and *S. gallinarium*. Note the presence of intact intestinal villi (arrow)

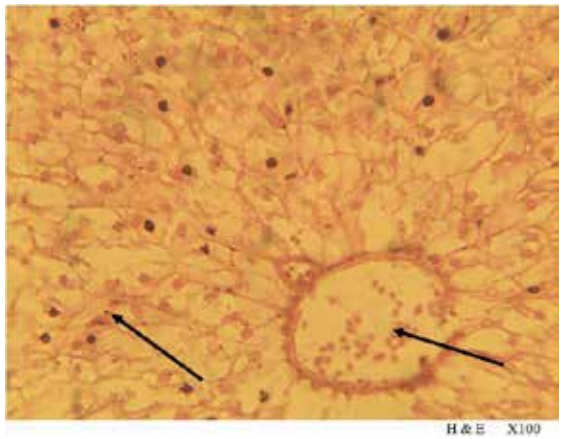

Figure 4: Photomicrograph of the liver of an African Catfish experimentally infected with *E. coli* and *S. gallinarium*. Note the presence of hepatocytes with cytoplasmic vacuoles and the central vein.

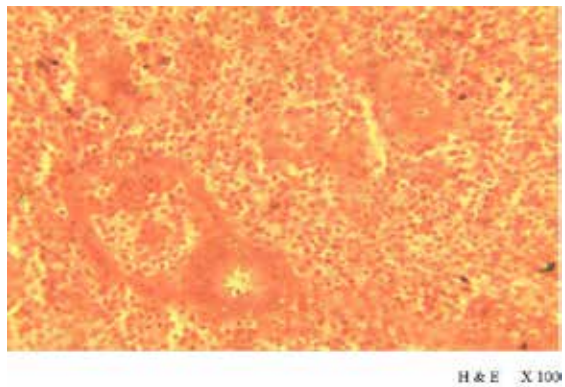

Figure 5: Photomicrograph of the spleen of an African Catfish experimentally infected with *S.gallinarium*. Note presence of multiple golden yellow depositions (*Haemosiderosis*)

1 and the tank water in groups A and C was turbid while that of groups B and D was clear. Five percent of the fish in group C died and mortality was observed on week four post infection' but no mortality was observed in groups A, B and D.

Effect of infection on growth rate

The results of mean weights are presented in table 2. By 1 week post infection the mean weight of group A was significantly ($P<0.05$) decreased compared with groups B,C and D. Starting from week 2 to 5, mean weight of groups A and C were significantly($P<0.05$)

lower than group D while it was comparable in groups C and B

Grossly, fish in group A were emaciated as shown in (Figure 1). The liver of fish in group A was pale and enlarged while areas of focal necrosis on the liver were observed in group B (Figure 2). There were no obvious skin lesions in any of the groups. There were also no visible lesions in the intestines among all the groups while there was vacuolation of hepatocytes and hepatic de*gener*ation in the livers of groups A and B, however there was multiple golden yellow depositions (*haemosiderosis*) on the spleens in group B (Figure 3, 4, and 5).

Discussion

The results of this study indicated that African catfish can be contaminated and infected with *E. coli* and *S. gallinarum*. This is in agreement with the work of several researchers who widely reported the presence of *E. coli* and Salmonella species in fish (Fattal *et al*., 1992; Silvakami *et al*., 1996; Quines, 1998). This implies that live fish may become a vehicle for *Escherichia coli* and Salmonella species from pond water during production and may carry the pathogen to processing facilities and/or retail food outlets. This present study has also shown that pond water can serve as a source of infection when they contain pathogenic organisms like *E. coli* and *S. gallinarum*. This is in agreement with studies of other researchers which revealed that fish cultured in a system that receives waste contaminated supply may harbour organisms and may serve as source of contamination to the fish. (Dalsgaard, 1998; Fapohunda *et al*., 1994; Silvakami *et al*., 1996 Alcaidae *et al*., 2005). The clinical signs (Sluggish movement, cuddling together, severe emaciation, poor growth rate, high morbidity, mortality and anorexia) observed in the infected fish from different experimental groups showed that they were diseased. These clinical signs were more pronounced in the group infected with *Escherichia coli* alone, than the groups infected with mixed *E. coli* and *S. gallinarum* or *S. gallinarum* alone. This may be attributed to the fact that *S. gallinarum* is host specific to birds and therefore may not cause severe infection in other animals like fish. High morbidity and low mortality (5%) were observed from the experimental groups. This differs from earlier studies by Nweke (2010), who reported anorexia, high morbidity and high mortality (100%) at five days post infection in catfish experimentally infected with Salmonella enteritidis. This may indicate that S. enteritidis is more pathogenic for fish compared to either *E. coli* or *S. gallinarum*. There were no skin lesion observed on fish in any of the experimental groups. This is not in agreement with previous work done by Udeze and Sowolu (2012) who reported peeling of skin in *C. gariepinus* inoculated with Salmonella species. This may be attributed to the reason that *E. coli* and *S. gallinarum* are enteric bacteria, they inhabit the intestine of animals, birds and fishes and can easily be isolated. They are not epitheliotropic and therefore may not cause obvious skin lesions in infected fish. The poor growth rate and poor weight gain observed in infected fish suggests that infection may lead to economic loss due to delayed maturity and marketing of the fish. The enlarged livers and necrotic livers observed in this study may be attributed to widespread destruction of tissues either due to the direct effects of the bacteria or their toxins absorbed from the fish blood streams. Histopathology results showed that there were no visible lesions in the intestines from the different experimental groups while there was vacuolation of hepatocytes and hepatic de*gener*ation in the livers of fish from the experimental groups A and B. The gross and histopathological lesions observed in the livers suggest that the bacteria (*E. coli* and *S. gallinarum*) may have a predilection site in the liver which predisposed the infected fish to hepatotoxic effects. However, the spleen from group B infected with *S. gallinarum* showed *haemosiderosis* (multiple golden yellow depositions). *Haemosiderosis* can be found in the spleen because the spleen helps in blood storage and in removal of senescent red blood cells but when it is found in a large numbers, it can be indicative of anaemia (Ragab *et al*., 2006).

Conclusion

This study demonstrated that the presence of *E. coli* and *S. gallinarum* can cause infection in African catfish (*C. gariepinus*) which can lead to adverse effects in their health status and production. This is the first description of the gross and histopathological effects of single and mixed experimental *E. coli* and *S. gallinarum* infection of *C. gariepinus* to our knowledge. The description of the clinical manifestations may be useful in diagnosis of infections caused by *E. coli* and *S. gallinarum* in African catfish.

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PRELIMINARY INVESTIGATION OF TOLL-LIKE RECEPTOR EXPRESSION AND *HAEMAGGLUTINATION* POTENTIALOF SELECTED PART OF THE REPRODUCTIVE TRACT OF GIANT AFRICAN LAND SNAIL (*ARCHACHATINA MARGINATA*) INFECTED WITH BACTERIA

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Abstract

A preliminary investigation was conducted on the toll-like receptor expression and haemagglutination potential in a selected part of reproductive tract of the Giant African Land Snail (GALs) (*Archachatina marginata*) infected with bacteria. A total of thirty (30) *Archachatina marginata* snails weighing between 150 g to 250 g were used for this study. Ten snails each were exposed to three treatments which included: T1 (0.2ml of 10^6 *Staphylococcus* aureus), T2 (0.2ml of 10^6 *Escherichia coli*) and T3 (0.2ml of distilled water). Total haemocyte counts were carried out at day 0, 2, and 3 with the aid of a haemocytometer. The haemagglutination potential of the albumen, ovotestis and hepatopancrease were assessed for confirmation of the presence of agglutinins before the infection procedure. Dissections of the snails were carried out ten days post infection and two vital organs (albumen gland and ovotestis) responsible for reproduction were removed for Toll-like receptor (TLR1) expression. Thereafter, DNA extractions were carried out on selected samples and PCR was carried out to confirm the expression. Results showed that TLR1 is present in both the Ovotestis and albumen glands of GALs. TLR1 was found to be expressed in both ovotestis and albumen gland subjected to bacterial infection. The levels of expression were found to be better in the ovotestis than in albumen. TLR1 was also observed to be more expressed in infected snails compared to the control group. The highest haemagglutination titres were recorded in both the Ovotestis and the hepatopancrease while the albumen gland recorded the least titre. It was also discovered that snail groups subjected to bacterial infection (*Staphylococcus* aureus and *Eschericia coli*) had higher haemagglutination titres compared to the control group given distilled water. It was also obvious that haemocyte counts declined at day 2 post-infection and returned to normal at day 3. It was concluded from this study that the expression of TLR1 in the Ovotestis and Albumen glands stimulate an immune response in the reproductive tract of GALs thus limiting bacterial activity on reproduction. The haemagglutination ability of these organs is also proof of the presence of agglutinins in them which play a crucial role in the innate immunity of the animal. Up-regulation of haemocytes at day 3 is also a sign of the ability to contain infections within a shortest possible period of time

Keywords: Toll-like receptor, Land snail, Reproductive tract, *Archachatina marginata*

Introduction

Giant African Land snails (GALs) are known to play significant roles in the life and culture of rural dwellers. They are known to be a very good delicacy among villagers, urban dwellers and among international hoteliers. Therefore, there is need for their rapid multiplication in order to meet both the local and international demand. However, the growth and multiplication of this animal faces many challenges. Prominent among these are the issues of climate change and bacterial invasion. This animal has a complex response to changes in environmental conditions. Pulmonate Land snails respond to unfavorable conditions such as drop in temperature and dehydrating periods by going into a state of inactivity called aestivation (Rizzatti and Romero, 2001; Storey and Storey, 1990). This state or period is a temporary shutdown physiological condition that limits reproduction. GALs are also exposed to bacterial pathogens, which could have obvious public health implications (Ugoh and Ugbenyo, 2013; Sodipe *et al*., 2013). There is need for effective understanding of their defensive mechanisms against these conditions.

The defensive system against pathogenic organisms is very important for the survival of Giant African Land snails (GALS). This animal has direct contact with the soil as it moves from one place to another with the skin directly exposed to bacterial invasion. Innate immunity is known to be well developed in invertebrates compared to vertebrates in which adaptive immunity is well established (Satake and Sekiguchi, 2012). Invertebrate innate immunity has been reported to consists of diverse pathogen recognition systems which include: hemolymph coagulation,lectin-mediated complement activation, antimicrobial peptides, and variable region-containing chitin binding proteins (Noaka, 2001; Khalturin *et al*., 2004; Iwanaga and Lee, 2005; Miller *et al*., 2007; Rast and Messiersolek, 2008; Bonura *et al*., 2009; Nonaka and Satake, 2010; Satake and Sasaki, 2010; Dishaw *et al*., 2011; Abiona, 2012). Recently, genome-wide analyses have established the presence of TLR and TLR-related genes in invertebrates. TLRs are type I transmembrane proteins which were found to play a vital role in mammalian host defenses via the innate immune system (Satake and Sekiguchi, 2012). TRLs are expressed in some vital organs which include: kidneys, ovarys and testes (Akira and Takeda, 2004; Dunne and O' Neill, 2005; Takeda and Akira, 2005). Their expression in these key organs is to protect the system from different levels of infection that may be caused by pathogens. TLR1, TLR 2, TLR 4, TLR 5 and TLR 6 are known to be responsible for recognition of extracellular microbial components (Akira and Takeda, 2004; Dunne and O' Neill, 2005; Takeda and Akira, 2005).

In the Giant African Land snail (*Archachatina marginata*), the key organs that are very important for reproduction and the wellbeing of the animal include: Ovotestis, albumen gland, and hepatopancrease (which has similar functions to the liver in mammals). All these organs needed to be protected especially during bacterial invasion in order not to compromise their functions. The presence of TLR in the innate immune response of GALS has not been established, therefore this study aimed at conducting preliminary investigations on the Toll-like receptor expression and haemagglutination potential of selected parts of the reproductive tract of the Giant African Land snail (*Archachatina marginata*) infected with bacteria.

Materials and Methods

Experimental Area

The research was carried out at the Snail Physiology Research Unit and Central Biotechnology Laboratory of the Federal University of Agriculture, Abeokuta, Ogun State. The location lies within the rainforest belt of Western Nigeria, latitude 7°N, longitude 3°2' E and the altitude 76 m.a.s.l. the climate is humid with a mean annual rainfall of 1,037mm, mean temperature of 34.7°C and mean relative humidity of 82% (Google Earth, 2017).

Materials

A total of thirty (30) *Archachatina marginata* snails weighing between 150g to 250g were purchased from a local market. Other materials included: Plastic cages (30cm by 40cm by 24cm), feeding trough, drinker, knife, concentrate, water, Vanier calliper, Pasteur pipette, marker pens and masking tape for proper identification, sensitive scale for weight determination, and a tape rule for measurement.

Snails and their Management

The plastic cages were washed before the commencement of the experiment, the first three weeks of the experiment was set aside as a period of acclimatization. Snails were fed *ad libitum*. Water was provided on daily basis. The drinkers, feeders and the cage were clean daily. The experiment lasted for eight (8) weeks.

Snail Grouping

The total number of snails used for the experiment was grouped into two:

i. Infected
ii. Non-infected (Control)

All snails in both groups were treated equally in terms of feeding and drinking water. The composition of feed used is given in table 1.

Bacteria Isolates And Inoculation Route

Two strains of bacteria were used for the study. The two bacteria were: *Staphylococcus* aureus (10^6) and *Escherichia coli* (10^6). These two bacteria were obtained from pure cultures processed at the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The inoculation route was the anterior part of the buccal mass.

Inoculation Procedure

A total of ten (10) snails were exposed to 0.2ml of 10^6 colony forming units (CFU) of each of the bacterial species (*Staphylococcus* aureus and *Escherichia coli*) thus making a total of twenty snails. For the control group, 10 snails were injected with normal saline using the same method as the infected group. Thereafter, albumen gland and ovotestis were collected to assess expression of TLR following infection with *Staphylococcus* aureus and *Escherichia coli*.

Experimental Design

The snails were randomly assigned to three treatments as follows:

Treatment 1: Infected with *Staphylococcus* aureus: 10 snails

Treatment 2: Infected with *Escherichia coli*: 10 snails

Treatment 3: Control: 10 snails (Distilled water)

Dissection of snails

The snails were dissected after day 10 post infection with the use of a dissecting set and the albumen gland and ovotestis were removed from the reproductive tract for molecular studies.

TLR Determination

DNA extraction

DNA was extracted from each sample, from the reproductive tract (ovotestis and albumen gland) using DNeasy blood and tissue kit following the manufacturer's protocol. All centrifugation was performed at temperatures of 15-25^{0}C.

TLR Primer and Polymerase Chain Reaction

PCR was performed using TLR primer under the following conditions: 95^{0}c for 2 minutes 30 cycles of 94^{0}C for 30 seconds, 58^{0}C for extension at 72^{0}C for 30 seconds followed by extended elongation at 72^{0}C for 5 minutes. The PCR products were separated on 1% Agarose gel and identified by ethidium bromide staining.

Table 1: Composition of experimental diets (g/100g)

Ingredients Table	Quantity (g)
Maize	50
Wheat offal	27.5
Groundnut cake	12.25
Soya bean meal	4
Bone meal	3
Oyster shell	3
Salt	0.25
Total	**100**

Table 2: TLR Primer

TLR	PRIMER SEQUENCES FORWARD /REVERSE	SIZE (bp)	Annealing Temp (°C)	Optimized annealing temp. for Nigeria Goat
1	CTGCCCATATGCCAAGAGTT/ AAACCAACTGGAGGATCGTG	421	58	56.3

Source: Menzies and Ingham, 2006

Agarose Gel Electrophoresis

After the PCR, the amplified fragments were electrophoresed on 1% (w/v) agarose gel at 120 volts for one hour. Alongside the sample fragments, a molecular size marker with different fragment sizes was electrophoresed on the same gel. The inclusion of a marker was important to estimate the size of the fragments as a confirmation of having amplified the right DNA fragment.

Visualization of PCR products

The detection of the amplified fragments was done under UV light using a transilluminator. The presence of the fragments of interest was verified and then a photograph was taken showing samples and markers in their respective lanes.

Total haemocyte count

Total haemocyte counts were carried out on days 0, 1, and 2 via haemolymph with the use of haemocytometer. Haemolymph was collected from representative group of infected and non-infected snails. A dilution of 1:19 of haemolymph and 5% eosin solution from both groups were mixed and loaded into an improved haemocytometer and cell counts carried out.

Preparation of materials for haemagglutination

Fifty milligrams (50 mg) of albumen gland, hepatopancrease and ovotestis harvested after dissection from the infected groups of snails were dissolved in two milliliters (2 ml) of phosphate buffered saline (PBS) and macerated. After maceration of each selected part, 100µl each from the bulk solution was used for haemagglutination.

Haemagglutination test

One hundred micro-litres (100µl) of haemolymph and bulk solution from albumen gland, hepatopancrease and ovotestis from the infected groups of snails were separately serially diluted with 0.85% of Phosphate Buffer Saline (PBS) at a pH of 7.2. Diluted mixtures were aliquoted into micro titre plate wells at 100µl per well. Thereafter, an equal volume of 2% sheep erythrocytes was added. The plates were covered and gently mixed. After mixing, incubation was carried out at room temperature for 30, 60, 90, 120 and 150 minutes. Two wells were allocated for the negative and positive controls. The negative control was made up of PBS and sheep red blood cells while the positive control consisted of pure lectin (Canavalia ensiformis) and sheep red blood cells.

Results

Plate 1 and plate 2 show the pictures of the gel electrophoresis on the optimization of DNA and TLR1 respectively. The difference observed in the band thickness is due to the treatment of the samples. This result shows that DNA is expressed in the selected reproductive parts (ovotestis and albumen gland). Expression was found to be better in the ovotestis compared to albumen gland. TLR1 was more expressed in the reproductive tract of the snails infected with S. aureaus and *E. coli* compared to the uninfected control snails (2).

Figure 1 shows the effects of bacterial infection on the haemagglutination titres of selected parts of the reproductive tract of the Giant African Land snail (A. marginata). Haemagglutination titres were higher in both the hepatopancrease and Ovotestis compared to the albumen gland that had the least titres. The effects of bacterial species on haemagglutination titre of Giant African Land snail (A. marginata) is shown in Figure 2.

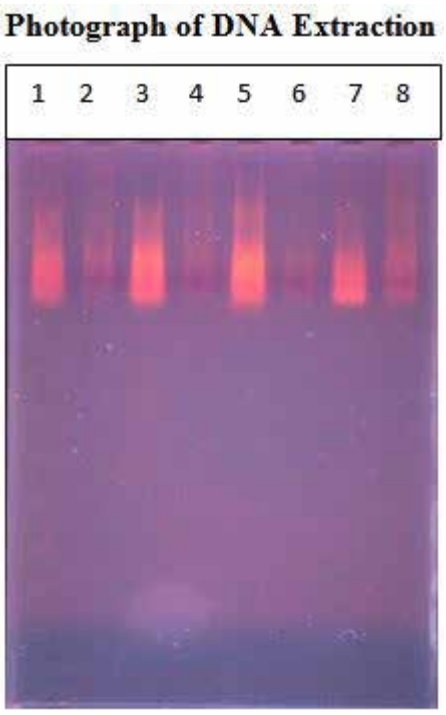

Plate 1: DNA of different selected parts of the reproductive tract exposed to bacteria

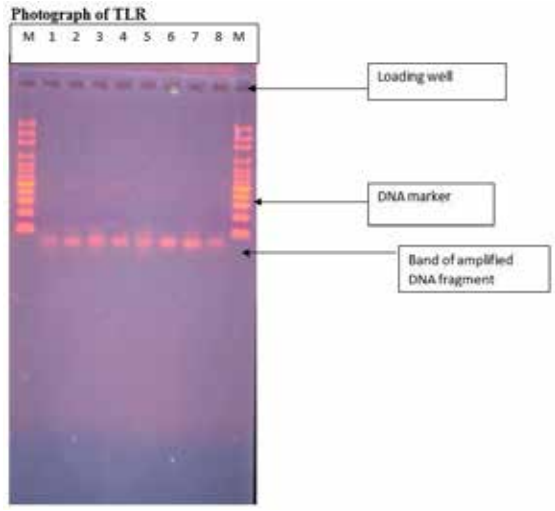

Plate 2: TLR1 expression in selected parts of the reproductive tract of *A. marginata* exposed to two species of bacteria

KEY:
1 - OvotestisStaphylococcus aureus, 2- Albumen gland Staphylococcus aureus, 3- Ovotestis Escherichia coli, 4- Albumen gland Escherichia coli, 5- Ovotestis Normal saline, 6- Albumen gland Normal saline, 7- Ovotestis Escherichia coli, 8- Albumen gland Escherichia coli

Identities of TLR1 among selected organs based on treatment given

In plate 1 the DNA was more expressed in the ovotestis compared to the albumen gland.

In plate 2 TLR1 was expressed in the *A. marginata* organs infected with bacteria.

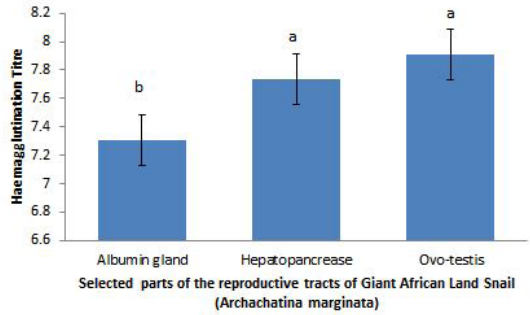

Figure 1: Effect of bacterial infection on *haemagglutination* titres of selected parts of the reproductive tract of the Giant African Land snail (*A. marginata*)

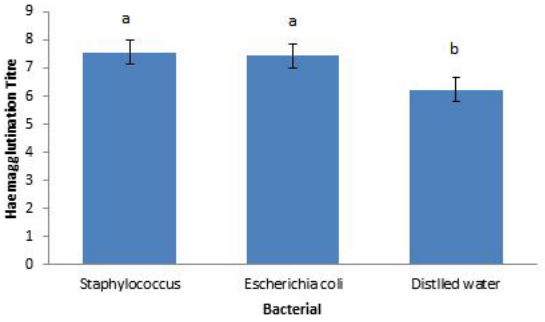

Figure 2: Effects of bacterial species on *haemagglutination* titres of the Giant African Land snail (*A. marginata*)

Snails exposed to *Staphylococcus* aureus and Eschericial coli had higher haemagglutination titres (7.56 vs 7.45) compared to the uninfected snail that had distilled water (6.22).

Figure 3 shows the effects on the total haemocyte counts of the Giant African Land snail (A. marginata) before and after infection with bacteria. Before infection at day one, the haemocyte count was at 12.83 $\times 10^3$, at day two post infection, it declined to 4.74 $\times 10^3$ and peaked on day three at 13.16 $\times 10^3$.

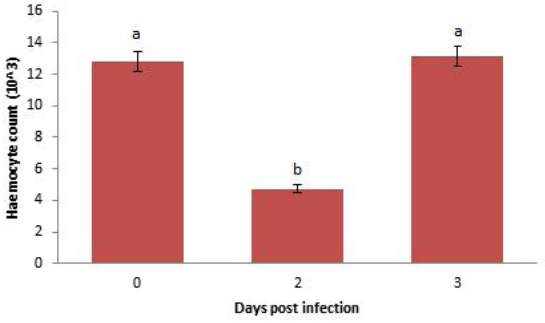

Figure 3: Total haemocyte counts of the Giant African Land snail (*A. marginata*) before and post infection with bacteria

Discussion

The results of the present study indicated that injection of *Staphylococcus* aureus and *Escherichia coli* into the reproductive tract of GAL could have induced or constituted the TLRs which recognize the proteins of the invading bacteria owing to the fact that the expression of TLRs can either be constitutive or induced according to Rodríguez-Martínez *et al.* (2011). It is well known that Pathogen-associated molecular patterns (PAMP) can induce the over-expression of its specific TLR because of its specificity in nature and actions, and the most well studied example is lipopolysaccharide (LPS), which can over-express TLR-4 (Faure *et al.*, 2001). Rodríguez-Martínez *et al.* (2005) reported that LPS from *Escherichia coli* over-express TLRs from 1 to 9. Results from another study observed that peptidoglycan (PGN) from *Staphylococcus* aureus acts as the most potent inducer for TLRs, Rodríguez-Martínez *et al.* (2011). The scope of the present study did not cover differences of PAMP recognized by TLRs. However, the results indicated the presence of TLR1 in both the ovotestis and albumen gland of the Giant African Land Snail. Meanwhile literature documented that over expression is not exclusive of a specific TLR, but has been observed that the induction with a particular PAMP can induce the expression of several TLRs (Otte *et al.*, 2003). The observation of the present study is an indication that TLRs is conserved in the selected segments of the reproductive tract. This was also evidence that these two organs can be of immune importance. It is also possible that they may produce other substance(s) that could assist in the fight against infections or diseases (Zhang *et al* 2007; Daffis *et al.*, 2008), for example, TLR2, has been reported to function as the transmembrane component involved in the detection of staphylococcal lipoteichoic acid and phenol-soluble modulin and is involved in the synthesis of inflammatory cytokines by *Monocytes*/macrophages in response to these components (Fournier and Philpott, 2005). TLR2 was reported to detect various specific components of pathogens. However the mode of action by which this TLR carry out its functions recognizing such a wide spectrum of stimuli is not clear but has been associated to cooperation of TLR2 with other receptors. The high levels of expression in the infected groups compared to the non-infected group indicated that the quantity of TLRs produced during infection is high as compared

to non-challenged condition which further shows the state of preparation for protection. In the natural environment, Snails come into contact with diverse bacteria which result in contamination during open circulation (Ugoh and Ugbenyo, 2013). However, the results of the present study provide an indication of the mechanisms of protection against bacterial infections. Therefore, TLRs can provide a host with versatility through the recognition of a wide range of pathogenic microorganisms; TLR may require a different level of organism burden for a given pathogen to reach the critical threshold for activation (Vazquez-Torres *et al*. 2004).

The haemagglutination results of the selected parts of the reproductive tract in this study confirmed that haemagglutinins are present in the hepatopancrease, the ovotestis and the albumen gland. The higher haemagglutination titres recorded in the hepatopancrease and ovotestis are a further indication that these two organs have the highest proportion of agglutinins present in them. Sanchez *et al*. (2006) reported the presence of N-acetylgalactosamine (GalNAc) binding lectin in the albumen gland of the roman snail called Helix pomatia agglutinin (HPA). With this report, it is certain that the presence of agglutinins in these three selected selected must have specific roles. Furthermore, Sanchez *et al*. (2006) arrogated a protective role to agglutinins especially that of protection of fertilized eggs from bacteria since the albumen gland is responsible for deposition of perivitelline fluid during egg formation. The presence of agglutinins in this organ forms part of the innate immunity system of this animal. Therefore, the presence of agglutinins in higher proportions in both the hepatopancrease and ovotestis is a further indication of protective role played by these organs which may be connected to both reproduction and other regulatory roles that may involve hormonal activities.

The higher haemagglutination titres recorded with both *Staphylococcus* aureus and *Escherichia coli* compared to the control (distilled water) is also an indication that bacterial invasion of snail systems triggers the production of lectin for effective bacterial binding. This is also a confirmation that there are sugar-lectin binding sites in both bacteria which sustain the process of agglutination. Bacteria are reported to have surfaces through which they attach to lectin via sugar moieties (Sharon, 1987). Mannose, galactose and N-acetylglucosamine are some of the sugars through which these bacteria bind. Jin *et al*. (2018) reported high binding affinity of *Canavalia ensiformis lectin* to carbohydrates on bacterial cell surfaces thus inhibiting biofilm formation of the foodborne pathogens enterohemorrhagic *Escherichia coli* and Listeria monocytogenes.

Haemocytes are categorized into hyalinocytes and granulocytes and are responsible for phagocytosis of parasites, pathogens, and foreign particles (Kennedy *et al*., 1996; Tiscar and Mosca, 2004). Hemocytes are also known to mediate cellular internal defence in molluscs via the accumulation and detoxification of chemical toxicants, phagocytosis and encapsulation of invading foreign, biological material (Bayer, 1973; Harris,1975; Van der Knaap *et al*., 1981; Matozzo *et al*., 2001; Chu, 2000; Canesi, 2002; Fisher, 2004). The observed reduction in the haemocyte count at day two (2) post infection indicated that bacterial toxin had been released into the system of snails immediately after infectionand this compromised the number of haemocytes. This observation is similar to what is obtainable in vertebrates as white blood cell numbers decreased immediately after infection. The increase in haemocytes seen at day three (3) post-infection was a reflection of the mass production of haemocytes to eliminate the bacteria via cell mediated responses. This process may also involve the production of agglutinins and antimicrobial peptides to further eliminate the invaders (Richards and Renwrantz, 1991; Abiona *et al*., 2012)

Conclusion

The results of the present study confirmed that TLRs is expressed in the ovotestis and albumen gland of the Giant

African Land Snail (*Archachatina marginata*) thus confirming the roles of these organs in immune mechanisms. The TLR presence also suggests that reproductive activity may not be hindered by both *Staphylococcus* aureus and *Esherichia coli* infections.. The presence of agglutinins in the ovotestis and albumen gland is also a further proof that these organs are protected by the innate immune system. Down regulation at day2 and up-regulation in the number of haemocytes at day 3 back to initial number is also a reflection of the ability of the innate immune system to contain infection within the shortest possible period of time.

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VILLAGE POULTRY PRODUCTION, HEALTH AND MANAGEMENT SYSTEM IN BENUE STATE, NIGERIA

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Abstract

Most rural communities in Nigeria keep village poultry. These birds are kept with minimal input of resources and are considered by most smallholders as supplementary to the main livelihood activities. A study on village poultry production, health and management system was conducted in 24 communities of six Local Government Areas (LGAs) of Benue State. Data were collected through interviews using structured questionnaires, group discussions with key informants and by direct observation. The results showed that the main management system used by village poultry farmers (VPF) was the free range system 92.9%. Most of the poultry farmers (95.6%) provided housing for their birds, some used their kitchens (40.2%) to house birds, about 32.6% used thatched houses. The study showed that 49% gave feed supplements to their birds in the mornings, 13.2% gave it in the evenings. The feed supplements given to birds included guinea corn (29.6%), maize (14.3%) and household leftovers. The main source of drinking water for the birds was from the community well (40.8%) and water from the river (35.7%). Predators (52%) and theft (22.4%) were identified as the commonest causes of losses in local poultry. About 42.0% of the village poultry farmers would eat sick birds, 19.0% (5/98) would use local treatment, while about 36.9% (35/98) would seek veterinary help. The weekly market was where most of the farmers (62.2%) sell their birds. About (81.6%) of the poultry farmers who participated in the study had some knowledge of poultry diseases with Newcastle disease ranking highest in terms of outbreaks and mortality. Women played a major role in village poultry development through ownership (61.2%) of the flocks and the provision of labor. The study concluded that the productivity of the village poultry in Benue State was low and thus calls for appropriate interventions focused on the improvement of feeding, housing and health care.

Keywords: Local poultry, free range, household, production, Benue state

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Introduction

In many developing countries, including Nigeria, chicken stands out as the most common livestock owned by many rural families. These village chickens play many vital roles in the lives of poor families. They provide meat and eggs, food for special festivals, offerings for traditional ceremonies and petty cash for the purchase of medicines and payment of school fees (Alders and Spradbrow, 2001). The importance of village poultry production in the national economy of developing countries and its role in improving the nutritional status and incomes of many smallholder farmers and landless communities has been recognized by various scholars and rural development agencies over the last few decades (Moges *et al*., 2010; Melesse *et al*., 2011). Village poultry makes the greatest contribution to the supply of meat and eggs for the average Nigerian, contributing annually about 89% of total poultry meat and over 25% of total poultry eggs consumed in the country (Sonaiya *et al*., 1999).

In nearly all African countries, poultry production in rural areas is predominantly based on free range systems utilizing indigenous types of domestic fowl (Kitalyi, 1998). The system is characterized by a family ownership of the birds. The birds are left to scavenge to meet their nutritional needs. The feed sources vary depending on local conditions and the farming system. Housing may not be provided and where this is done, local materials are usually used for construction (Atunbi and Sonaiya, 1994). Management is minimal with some variations of gender roles in the activities. The health of the birds are not guaranteed because there are no disease control programmes (Achiempong, 1992). The birds are exposed to many disease conditions and parasites are also prevalent due to favourable conditions. In spite of the low-input by rural farmers for their production, free-range birds play many socio-economic roles (Kitalyi, 1998; Permin and Hansen, 1998). The productivity of village chickens is known to be very low (Gueye, 1998). According to Aini (1999), poor reproductive performances, diseases and high costs of feed are the main constraints of rural poultry production.

In Nigeria, mortality of indigenous chickens under the free-range system was very high due to diseases, poor management, poor breeding systems and malnutrition (Dipeolu *et al*., 1998). Similarly, Alders *et al*, (2009) revealed that high mortality and high parasite load due to inadequate housing and health care are problems of extensive poultry production. The predominance of the village poultry in the Nigerian poultry industry is a good indicator that these birds deserve more attention for improved performance. Considering the significance of this production system in the improvement of rural livelihoods, it is important that to further study it to identify possible areas of improvement and strategic entry points.

Materials and Methods

Study area

The study was conducted in Benue State located in the north central zone of Nigeria. The state lies within longitude 7° 47' and 10° 0' East, Latitude 6° 25' and 8° 8' North of the Equator and shares boundaries with Nasarawa, Taraba, Cross River, Enugu and Kogi States and the Republic of Cameroun. Benue State has an estimated total poultry population of 6,735,041 (Adene and Oguntade, 2006).

Sampling technique

Six Local Government Areas were randomly selected which included Gboko, Katsina Ala, Kwande, Makurdi, Oju and Otupko LGAs. A total of 24 villages comprising of four villages selected from each LGAs were selected for the study. In each of the selected villages, four households were sampled based on their consent and readiness of the village poultry owners to participate in the study.

Assessment of village poultry health, production and management system

The assessment of the village poultry production was undertaken through the use of structured questionnaires. The questionnaires were designed and first pretested before they were administered to the poultry farmers.

The questionnaires were administered and interpreted into the local languages of Tiv and Idoma where necessary. Detailed information was obtained on the management practices, housing, health and sale of stock and problems prevailing in local poultry production in the study area. A total of 98 respondents participated in the study

Data analysis

The data obtained from the questionnaires were analyzed by descriptive statistics using the Statistical Package for Social Sciences version 17.0 program (SPSS Inc. Chicago, IL, USA). The frequency and percentages were calculated.

Results

The main management system used by the local poultry farmers was the free range system 92.9% (91/98) while 7.1% (7/98) used a semi intensive system (Figure 1). The study showed that 49% (48/98) of the village poultry farmers gave feed supplements to their birds in the mornings, 9.2% (9/98) in the evenings while about 37.8% (37/98) gave feed supplement both in the mornings and in the evenings. The feed supplements included guinea corn 29.6% (29/98), maize 14.3% (14/98) and kitchen leftovers. Most of the farmers (95.6%) provided housing for the birds while (4.4%) did not provide housing. Some of the farmers used the kitchen (40.2%) to house birds, about (32.6%) used thatched houses, (21.7%) used zinc houses while (5.5%) housed birds in mud houses (Figure 2). The study revealed that most of the village flocks 61.1% (58/95) were owned by wives with about 20.0% (19/95) being owned by the husbands, 14.7% (14/95) were owned by the children, wives and husbands while 4.2% were jointly owned by the husband and wife. About 51.0% of the flocks were cared for by the wives and the children (Table 1). Birds of prey 52% (51/98) were the commonest cause of loss in village poultry in the study area. Other causes of loss included theft 22.4% (22/98) and rodents 3.1% (3/98) (Table 2). About 42.0% (40/98) of the village poultry farmers would eat their birds when they are sick, 19.0% use local treatments for the birds and 2.1% (2/98) would rather allow them to die. However, about 36.9% (35/98) would seek veterinary help. The main source of drinking water for the village poultry was from the community well 40.8% (40/98) and water from the river 35.7% (35/98). Weekly markets 62.2% (61/98) were where most farmers sell their birds while 10.2% (10/98) sold in the daily markets and 11.2% (11/98) sold to poultry traders. However, 2.0% (2/98) sold to other households in the village while 14.2% (14/98) sold poultry in both weekly markets and to poultry traders. Most of the farmers 56.1% (55/98) do not buy new birds but breed them. However, 27.6% (27/98) buy new birds from the market while others buy from households in the village.

The disease that was reported to rank first in terms of outbreaks and high mortality was ND with (82.6%) followed by avian influenza (9.8%) and fowl pox (7.6%) (Figure 3). Most of the farmers 81.8% (72/88) do not vaccinate their birds against ND, only 18.2%

Table 1: Ownership and care of village poultry flocks by some farmers in Benue State, Nigeria.

Family members	Care of flocks % respondents	Owners of flock % respondents
Husband/wife/children	0.0	14.7 (14)
Husband/children	1.0 (1)	0.0
Wife/children	51.0 (50)	0.0
Husband	12.2 (12)	20.0 (19)
Wife	32.7 (32)	61.1 (58)
Husband/wife	3.1 (3)	4.2 (4)
Total	**100 (98)**	**100 (95)**

Table 2: Common causes of losses in village poultry production in Benue State, Nigeria.

Cause of loss	No. of respondent	Percentage
Accidents	3	3.1
Birds of prey	51	52.0
Birds of prey/rodents	2	2.0
Birds of prey/rodents/theft	4	4.1
Birds of prey/accidents/rodents	5	5.1
Dogs/rodents	8	8.2
Rodents	3	3.1
Theft	22	22.4
Total	**98**	**100**

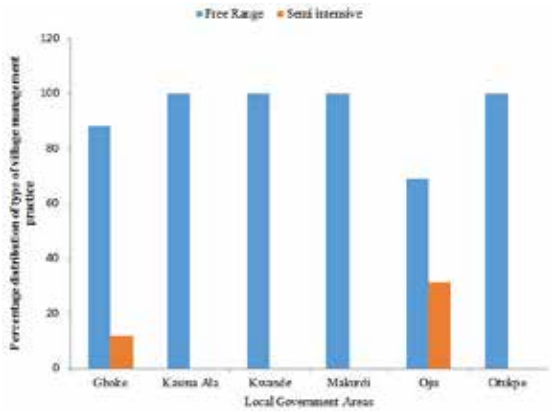

Figure 1: Percentage distribution of type of management system used by village poultry farmers in some Local Government Areas in Benue State, Nigeria.

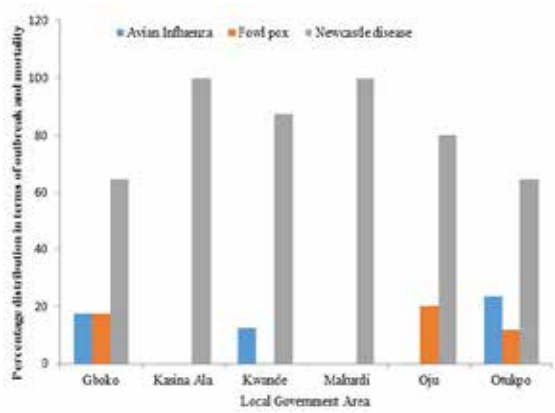

Figure 3: Distribution by Local Government Areas of the poultry disease that rank first in terms of outbreak and mortality in Benue State, Nigeria.

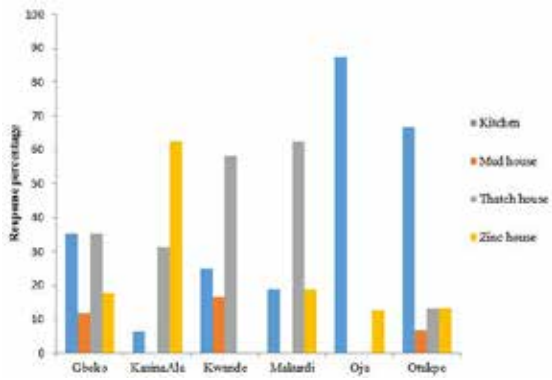

Figure 2: Type of village poultry house used by owners in some local Government areas in Benue state, Nigeria

(16/88) reported vaccination. On measures necessary to prevent disease outbreaks, 30% of the farmers agreed that more education and awareness on poultry diseases would help to prevent disease outbreaks on their farms while (15%) and (14%) reported that early disease detection and regular visits from veterinary officers. (Table 3) would be necessary.

Table 3: Knowledge of village poultry farmers on disease prevention and control in Benue State, Nigeria.

Cause of loss	No. of respondent	Percentage
Clean feed and water	11	11.2
Early disease detection	15	15.3
High compensation for culled birds	2	2.0
More education/awareness on disease prevention	30	30.6
Safe source of birds	2	2.0
Available vaccine	9	9.2
Someone to advice farmers when birds are sick	10	10.2
Reduce contact between birds from each household	5	5.1
Regular visit from veterinary officers	14	14.3
Total	**98**	**100**

Discussion

The main management system used by the respondents was the free range system. Free-range birds do not receive sufficient feed but survive through scavenging. The feed resource base for scavenging birds is limited and varies with seasonal circumstances such as rainfall, cultivation, harvest and crop processing. During the rains, they feed on abundant animal protein by picking up worms, snails and insects (Alders *et al*., 2009). During grain harvests, birds can usually scavenge on enough energy feed but not in the dry season. In spite of the fact that village poultry scavenge around homesteads and surroundings the study revealed that farmers gave supplementary feedstuff to their birds. Guinea corn, maize and food scraps constitute the major supplements given. Improved feeding as part of the management system for poultry improves the disease resistance of birds to infection (Marvale, 2000). The majority of farmers provided supplementary feed twice a day, in the morning before going to farm and in the evening when they are back from farms. The majority of farmers also provided housing for their birds. The Provision of housing, improved feeding and *general* management have been reported to reduce the incidence and severity of Newcastle disease in birds (Marvale, 2000). Similarly, Nwanta *et al*. (2006) reported that the provision of houses protects chickens and chicks from predators, diseases and provide warmth to birds during cold weather. The study also revealed that most of the village flocks were owned by women. This is similar to the report of Kitalyi (1998) and Mapiye and Sibanda (2005) who reported that ownership of village chickens is by women more than the men and added that women dominate most domestic activities and thus have control over domestic chickens. The participation of women in rural poultry improvement programs contributes to human development both by increasing access for rural women to income and by empowering them through provision of microcredits and training and thus increasing production efficiency (FAO, 1988; Gueye, 2005). Birds of prey were the commonest cause of loss in village poultry in the study area. Other causes reported by the poultry owners included thefts and rodents. This is in agreement with the reports of Hassan *et al*, (2012) in Nasarawa State who reported that apart from disease as a biggest single cause of losses in village poultry other causes come from predators, stealing and parasites. Adene and Oguntade (2006) also reported that, village poultry suffer losses from predators and diseases caused by viruses, bacteria and parasites. The predators include primarily birds of prey such as vultures, which prey only on chickens and wild mammals such as cats and foxes, which prey on mature birds as well as chicks (Tadelle *et al*., 2000). The main source of drinking water for the village birds were from the community well and river.

These sources of water were also used as drinking water by the villagers. The implication of this is that wild birds and ducks which are reservoirs of avian diseases such as Newcastle disease and Avian influenza are attracted to open water bodies. This could result in the spread of diseases from infected or carrier birds to susceptible birds when the water is contaminated with secretions and faeces. The provision of potable water has been reported by Nwanta *et al.* (2006) to reduce the chances of infection particularly when given with food immediately after the birds are released in the morning to scavenge.

The study revealed that village poultry farmers used various methods to handle sick birds. Some used local remedies, others slaughtered and ate sick birds while others sold or gave them as gifts. The use of such treatments may be attributed to illiteracy, poverty, lack of knowledge of basic health and management practices and lack of institutional interventions. Ethno veterinary practices among farmers included the addition of ground dry pepper to drinking water or feed, sliced onions in drinking water, use of bark, leaves or seed of some plants in drinking water and palm oil for fowl pox. The farmers believed that these local treatments work for them as it improves the health of their birds. The majority of the farmers obtained breeding stocks from and sell off their birds at the weekly live bird markets (LBMs). The LBMs comprise of a pool of sick and healthy birds of different species and types enhancing disease introduction and transmission (Abah *et al*., 2017). Whenever such birds were not sold at the LBMs, they were returned to households and mixed with other birds. This practice could lead to disease outbreaks due to exposure to infectious agents in the LBMs.

Knowledge of poultry disease was high among the farmers with Newcastle disease (ND) ranking first in terms of the recognition of clinical signs and reports of high mortality during outbreaks. Many studies indicate that ND is the main cause of mortality in village poultry (Guèye, 1998; Sonaiya *et al*., 1999). Despite the fact that most farmers were aware of ND, the majority do not vaccinate their birds against ND. This could be due to the fact that conventional vaccines are unsuitable for sustained use in the village poultry sector because of their cost, large dose presentations, transportation and need for maintenance of a cold chain as reported by Alders and Spradbrow (2001) and (Musa *et al*., 2010). Similarly, reports of Alders *et al*, (2009) revealed that high mortality and high parasite loads due to inadequate housing and health care are among the problems of extensive poultry production.

Conclusion

The results of this study showed that local poultry in the Benue State of Nigeria were raised under a free range management system. Women and children were the main providers of care for birds and women owned most of the village flocks. Newcastle disease and birds of prey were the major causes of mortality and losses in village poultry and most of the village poultry farmers do not vaccinate their birds against ND. The study recommends that extension agencies should be mandated to disseminate education and improved technology that will stimulate local poultry production in the study area. Capital can be channeled to local poultry production through the provision of microcredit loans and the formation of cooperative societies. Women are the most suitable target group through which improvement strategies can be channeled. Extension service workers should also focus on educating women on poultry diseases to help prevent outbreaks. Farmers should source their breeding stock from reputable sources that have no disease problems among their flocks instead of the open market where both sick and healthy birds are mixed up. This will help in reducing disease outbreaks and mortality. Drugs and vaccines should be provided for local poultry production at affordable prices to improve the productivity of village poultry flocks.

Acknowledgements

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HAEMATOLOGICAL AND SERUM BIOCHEMICAL RESPONSES OF WEST AFRICAN DWARF BUCKS TO DAILY DRENCHING WITH *AFRAMOMUM MELEGUETA* SEED EXTRACT

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Abstract

This study was conducted to evaluate the haematological, blood fatty acid and serum biochemical constituents of West African dwarf bucks drenched with *Aframomum melegueta* extracts. Sixteen West African Dwarf Bucks aged between 10 to 12 months old were used. The bucks were randomly assigned into four groups consisting of four animals per group. Treatment 1: (Control, 0 mg *A. meleguata* seed extract (AMSE)/kg BW but received saline, Treatment 2: (25 mg MSE)/kg BW; Low dose), Treatment 3: (50mg *A. meleguata* seed extract (AMSE)/kg BW; Medium dose), Treatment 4: (75mg *A. meleguata* seed extract (AMSE)/kg BW; High dose). Feed and water were given *ad libitum*. All the data *generat*ed in this study were subjected to analysis of variance in a complete randomized design (CRD) and regression analysis. Drenching West African Dwarf bucks with different dosages of *Aframomun melegueta* seed extract (AMSE) did not influence (P>0.05) their haematological, blood fatty acid and serum biochemical parameters three hours post treatment except the red blood cells.. the *Cholesterol* content and high density *Lipoprotein* (HDLP) reduced significantly (P<0.05) in the three hours post-treatment except in the control group. Low density *Lipoprotein* (LDLP) was not influenced (P>0.05) by AMSE drenching in WAD bucks although there was a *general* reduction in the LDLP values in all the treatments but more pronounced in animals drenched with 25mg/BW AMSE. The regression models relating the drenching dosage of AMSE to haematological and serum biochemical parameters of WAD bucks showed that the Coefficients of determination (R2) values for the linear, quadratic and allometric models followed the same trends. The study concluded that the haematological, blood fatty acid and serum biochemical constituents of West African dwarf bucks drenched with *Aframomum melegueta* extracts were not significantly affected after three hours post-treatment.

Key words: haematological parameters, serum biochemical constituents, blood fatty acid Constituents, West African dwarf bucks, *Aframomum melegueta* extracts.

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Introduction

Aframomum melegueta (commonly known as Grains of Paradise, Melegueta pepper, alligator pepper, Guinea grains, fom wisa or Guinea pepper) is a species in the ginger family, Zingiberaceae. This spice is obtained from the ground seeds; it imparts a pungent, peppery flavour with hints of citrus (Daiziel, 1955; Beichner, 1961; Lock, *et al*., 1977; Isawumi, 1984 and Sugita, 2013). *Aframomum melegueta* falls among the plants with promising and effective anti-infective activity as presented by (Iwu *et al*., 1999). Its medicinal importance and property was also described to include anti - inflammatory and antimicrobial activities, to relieve dental pains, asthma and body weakness, enhance body activities and preservation of grains, carminative, peripheral analgesic activities diuretic, aphrodisiac, lactation aid, some mollucicidal and repellant properties (Addae-Mensah and Aryee, 1992; Iwu, 1993; Umukoro and Ashorobi, 2001; Ukeh *et al*., 2011 and Sugita, 2013). There is enormous information on the medicinal, antimicrobial and chemical composition of A. melegueta according to Iwu, 1993; Lajide *et al*., 1995; Taire *et al*., 1999 and konning *et al*., 2004. The inclusion of the seeds of A. melegueta in the diet has been reported to be responsible for the cardiovascular health of gorillas in the wild (Dybas and Ilya, 2007). It has also been reported that A. melegueta has been used as a purgative, anthelmintic and haemostatic agent (Ilic *et al*., 2010; Akendengue and Louis, 1994, Strisvastava and Mustafa, 1998). Okwu, 2001 reported that A. melegueta contains 0.000437g of *Calcium*, 0.00002886 g of Iron and 0.000194 g of Magnesium as well as Vitamins A, B, C, D and E.

The presence of phenolic compounds in the seed of A. melegueta indicates that this plant is an antimicrobial agent because phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (Okwu, 2001). Extracts from the seed of .A melegueta have potent antiseptic or bactericidal properties and have therefore, been used in treating wounds and in the prevention of infections such as intestinal infections and infestations, to calm indigestion, heartburn, and diarrhea (Okwu, 2004).

A study on the effects of A. melegueta and normal diet on the Albino rat showed that the serum total *Cholesterol*, *Triglyceride*, and high density *Lipoprotein* (HDL) *Cholesterol* concentrations decreased in rats placed on a normal diet with guinea pepper as compared to the control (Mohammed *et al*., 2013). Many studies have been carried out on A. melegueta extract; it's inhibitory activity, antioxidant properties, its interactions with hormones, organs and even cancer in different animal species. But there is a paucity of information on the effect of A. melegueta extract on the serum biochemical properties of WAD bucks. Since A. melegueta has antimicrobial and anthelmintic property, there is a high possibility that it enhances the haematological parameters and serum biochemical constituents. Hence in this study, the effects of daily drenching of WAD bucks with extracts of *Aframomum melegueta* on the haematological parameters and serum biochemical constituents were investigated.

Materials and Methods

Experimental location

The experiment was carried out at the Goat Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Nigeria. The site is located in the derived savannah region on latitude 7o10 N and longitude 3o2 E, with a mean annual temperature of 32oC and relative humidity of 82%.

Experimental Animal and Management

The experimental animals comprised of twelve West African Dwarf Bucks aged 10 to 12 months old. The goats were housed in a pen of 2m x 1m constructed using wooden material, with dwarf walls of about 1m high separating them and the whole pen was covered with wire-net to prevent unauthorized entry and facilitate ventilation. On arrival, the goats were acclimatized for about 2weeks before being

served the experimental diet. The goats were treated against ecto- and endo-parasites prior to the beginning of the study.

Experimental Design

The bucks were randomly assigned into four groups consisting of four animals per group. Treatment 1 (Control, 0mg *A. meleguata* seed extract (AMSE)/kg BW but received saline solution , Treatment 2 (25mg AMSE/kg BW; Low dose), Treatment 3 (50mg AMSE/kg BW; Medium dose), Treatment 4 (75mg AMSE/kg BW; High dose).

The bucks were fed on dry Panicum maximum ad. lib and on concentrates. Below is a table of the ingredient composition (kg/100 kg DM) of the concentrate diet fed to the West African Dwarf bucks.

Table 1: The ingredient composition (kg/100 kg DM) of the concentrate diet fed to the West African Dwarf bucks.

Ingredient (%)	Concentrate feed
Cassava peel	53.2
Corn bran	12.5
Wheat offal	4.0
Soybean meal	29.8
Common salt (NaCl)	0.5
Total	**100**

Data Collection

The animals were fitted with an indwelling jugular Catheter 24 hours before the commencement of the experiment. The bucks were stanchioned and blood was obtained from each animal at 10 minutes intervals for 3 hours before the animal was drenched with either saline solution or AMSE the pre-treatment period, period 1 and at 10 minute interval for another 3 hours after the saline solution or AMSE injection the post treatment period, period 2. The blood was collected the jugular vein into ethylene diamine tetra acetic acid (EDTA) and EDTA free tubes . The blood samples were stored on an ice pack prior to the harvesting of the plasma. The behavioral response of the experimental animals was observed for 15 minutes after the injection of the AMSE injection. An automated haematology analyzer was used to analyze the haematological parameters like packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), *Neutrophil*s (NEU), *Lymphocytes* (LYMP), *Monocytes* (MON) and eosonophils (EOS) and heamoglobin concentration (HB). The blood samples collected in EDTA free tubes were centrifuged to harvest the serum using a refrigerated (4°C) centrifuge at 3,000 rpm. The serum was stored at -20°C until it was analysed for biochemical parameters.

Determination of Serum Biochemical Parameters

Serum total protein and *Cholesterol* were determined spectrophotometrically according to the method of Tietz (1995) as described in Randox diagnostic kit manual. Serum *Albumin* and creatinine were determined according to the method of Grant *et al.*(1987) and the method of Henry (1964), respectively as described in the Randox diagnostic kit manual. Serum *Glucose* was determined spectrophotometrically using the method of Bartham and Trinder (1972).

Statistical Analysis

Data was analysed using one way analysis of variance in a completely randomized design. Regression analysis was used to predict the relationship between the serum biochemical parameters and the AMSE dosage

Results

Drenching West African Dwarf bucks with different dosages of AMSE did not influence ($P>0.05$) their haematological parameters three hours post treatment except for the red blood cells (Table 2).

The red blood cell (RBC) counts significantly decreased in the three hours post-treatment except in animals drenched with 25mg/kg BW AMSE which increased from 2.33 x 106/mm^3 to 2.40 x 106/mm^3. The highest reduction (0.67 x106/mm^3) was observed in bucks drenched with 50mg/kg BW. Effect of *Aframomun melegueta* on some serum biochemical constituents of West African Dwarf

Table 2: Effect of *Aframomun melegueta* on the haematological parameters of West African Dwarf bucks

Parameters	Drenching (mg/kg BW)				
	0	25	50	75	SEM
Packed cell volume (%)					
THBD	14.33	16.67	23.67	20.67	2.888
THAD	11.33	17.67	19.00	16.67	2.888
***Haemoglobin*(g/dl)**					
THBD	4.6	5.97	7.83	6.93	0.755
THAD	3.83	5.90	6.33	5.50	0.755
Red Blood Cell (106/mm^3)					
THBD	2.03^c	2.33^c	3.57^a	2.83^b	0.400
THAD	1.6^d	2.40^b	2.90^a	2.27^c	0.400
White Blood Cell (106/mm^3)					
THBD	7.33	10.93	7.63	4.5	2.768
THAD	9.07	6.87	6.33	5.10	2.768
***Neutrophil* (%)**					
THBD	38.67	23.33	33.67	27.33	4.685
THAD	31.67	32.33	37.00	37.67	4.685
***Lymphocytes* (%)**					
THBD	57.33	73.33	63.67	67.33	4.343
THAD	64.67	63.00	60.67	58.00	4.343
***Monocytes* (%)**					
THBD	4.00	2.67	2.67	4.67	0.839
THAD	3.00	4.00	2.00	3.33	0.839
***Eosinophil* (%)**					
THBD	0.00	0.33	0.00	0.67	0.461
THAD	0.67	0.33	0.33	1.00	0.461
***Basophil* (%)**					
THBD	0.00	0.33	0.00	0.00	0.136
THAD	0.00	0.33	0.00	0.00	0.136

^{a,b,c} Means on the same row with different superscripts are significant(P<0.005)
THBD- Three hour before drenching; THBA- Three hour after drenching; BW- Body weight

bucks at three hours pre-treatment and three hours post-treatment is presented in Table 3.

There was no significant (P>0.05) influence of AMSE drenching on all the serum biochemical parameters determined. The effect of AMSE on blood fatty acid constituents of West African Dwarf bucks at three hours pre-treatment and three hours post-treatment is depicted in Table 4.

Table 3: Effect of *Aframomun melegueta* on some serum biochemical constituents of West African Dwarf bucks

Parameters	**Drenching (mg/kg BW)**				
	0	**25**	**50**	**75**	**SEM**
Total Protein(g/l)					
THBD	6.50	6.73	7.50	6.73	
THAD	5.90	6.27	6.27	6.53	
***Albumin*(g/l)**					
THBD	2.55	2.60	2.50	2.47	
THAD	2.60	3.13	2.67	2.47	
***Globulin*(g/l)**					
THBD	3.95	4.13	5.00	4.27	
THAD	3.3	3.13	3.60	4.07	
***Glucose*(g/l)**					
THBD	54.00	55.00	53.50	51.33	
THAD	64.00	57.00	52.67	58.00	
Creatinine(g/l)					
THBD	1.70	1.83	1.30	1.20	
THAD	1.15	1.07	1.37	1.87	

Table 4. The effect of *Aframomun melegueta* on the blood fatty acid constituents of West African Dwarf bucks

Parameters	**Time of Sample collection**	**Drenching (mg/kg BW)**				
		0	**25**	**50**	**75**	**SEM**
Cholesterol(g/l)						
	THBD	2.48^{a}	2.15^{b}	1.09^{c}	2.15^{b}	
	THAD	3.25^{a}	2.08^{b}	1.06^{c}	0.55^{c}	
Low Density *Lipoprotein*(g/l)						
	THBD	32.23^{a}	29.33^{b}	25.33^{c}	24.17^{d}	
	THAD	30.73	23.71	24.53	22.75	
High Density *Lipoprotein*(g/l)						
	THBD	33.90^{a}	30.43^{b}	26.40^{c}	26.09^{c}	
	THAD	34.23^{a}	24.93^{b}	26.18^{b}	25.00^{c}	
Triglyceride(g/l)						
	THBD	3.98^{a}	2.13^{b}	1.92^{c}	2.13^{b}	
	THAD	2.63^{b}	1.81^{c}	2.67^{a}	1.80^{c}	

^{a,b,c} *Means on the same row with different superscripts are significant(P<0.005)*
THBD- Three hour before drenching;THBA- Three hour after drenching; BW- Body weight

The *Cholesterol* content reduced in the three hours post-treatment except in the control goats. The *Cholesterol* content reduced ($P<0.05$) from 2.15 g/l to 2.08g/l, 1.09 g/l to 1.06g/l, and 2.15 g/l to 0.55g/l, three hours after drenching the bucks with 25mg/kg BW AMSE, 50mg/kg BW AMSE and75mg/kg BW AMSE, respectively. Low density *Lipoprotein* (LDLP) was not influenced ($P>0.05$) by AMSE drenching in WAD bucks although there was a *general* reduction in the LDLP values in all the treatments but more pronounced in animals drenched with 25mg/kg BW AMSE. High density lipid protein (HDLP) reduced significantly ($P<0.05$) in all treatments at three hours post-drenching with AMSE except in the control group of bucks. The highest reduction in the serum HDLP was observed in bucks drenched with 25mg/kg BW at three hours post-treatment. From this study, drenching WAD bucks with 50mg/kg BW increased the *Triglyceride* content from 1.92 g/l at three hours pre-treatment to 2.67 g/l at three hours post-treatment while drenching the bucks with 25mg/kg BW and 75mg/kg BW produced a similar effect on the *Triglyceride* profile of WAD bucks.

Table 5 shows the regression models relating the drenching dosage of AMSE to the haematological parameters of WAD bucks. The Coefficients of determination (R2) values for the linear, quadratic and allometric models followed the same trends. In the linear model, the regression coefficient (b) showed that every 25mg/kg BW drenching of AMSE resulted in 0.053% change in PCV, 0.011 x106/mm3 in RBC, 0.033 % in *Neutrophil*s, 0.018 g/dl in *Haemoglobin*, 0.038 x 106/mm^3 in WBC and *Lymphocytes* and 0.001% in *Eosinophil*s and *Basophil*s. In the quadratic regression model, the c values indicated that the direction of change was downward (negative) for PCV, WBC, RBC, *Haemoglobin*, *Lymphocytes* and *Basophil*s while the direction of change was upward (positive) for *Neutrophil*s, *Monocytes* and *Eosinophil*s. In the allometric regression model, exponential changes of 0.198 (PCV), -0.156 (WBC), 0.249 (RBC), 0.205 (*Haemoglobin*), 0.037 *Neutrophil*s, -0.017 (*Lymphocytes*), -0.001 (*Monocytes*), -0.196 (*Eosinophil*) and -0.180 (*Basophil*) were observed. Except for *Eosinophil*s and *Basophil*s, the coefficient of determination (R2) values were very high indicating that the models were reliable in predicting the change in haematological parameters of WAD bucks drenched with AMSE.

Table 6 shows the regression models relating the drenching dosage of AMSE to the serum biochemical parameters of WAD bucks. The Coefficients of determination (R^2)

Table 5: Regression Model Relating the Level of Inclusion of *A. meleguata* Seed Extract to the Haematological Parameters.

Parameters	N	A	B	C	R^2
LINEAR MODEL					
Packed Cell Volume (%)	36	15.423	0.053		0.938
White Blood Cell (106/mm3)	36	11.526	-0.038		0.753
Red Blood Cell (106/mm3)	36	2.403	0.011		0.905
*Neutrophil*s (%)	36	28.878	0.033		0.921
Lymphocytes (%)	36	67.677	- 0.038		0.984
Monocytes (%)	36	2.956	0.003		0.812
Eosinophil (%)	36	0.644	-0.001		0.374
Basophil (%)	36	0.089	-0.001		0.067
Haemoglobin(g/dl)	36	5.112	0.018		0.939

Parameters	N	A	B	C	R^2
QUADRATIC MODEL					
Packed Cell Volume (%)	36	13.071	0.336	-0.004	0.955
White Blood Cell (106/mm3)	36	10.906	0.037	-0.001	0.756
Red Blood Cell (106/mm3)	36	1.956	0.065	-0.001	0.927
Neutrophils (%)	36	30.267	-0.134	0.002	0.923
Lymphocytes (%)	36	65.861	0.179	-0.003	0.984
Monocytes (%)	36	3.344	-0.044	0.001	0.825
Eosinophil (%)	36	0.811	-0.021	0.000	0.402
Basophil (%)	36	0.033	0.006	-0.000	0.122
Haemoglobin(g/dl)	36	4.323	0.113	-0.001	0.957
ALLOMETRIC MODEL					
Packed Cell Volume (%)	36	14.815	0.198		0.941
White Blood Cell (106/mm3)	36	11.414	-0.156		0.751
Red Blood Cell (106/mm3)	36	2.300	0.249		0.909
Neutrophils (%)	36	29.237	0.037		0.921
Lymphocytes (%)	36	67.148	-0.017		0.983
Monocytes (%)	36	3.059	-0.001		0.812
Eosinophil (%)	36	0.710	-0.196		0.377
Basophil (%)	36	0.064	-0.180		0.057
Haemoglobin(g/dl)	36	4.909	0.205		0.943

Table 6: Regression Model Relating the Level of Inclusion of *A. meleguata* Seed Extract to the Serum Biochemical Parameters.

Parameters	N	A	B	C	R^2
LINEAR MODEL					
Total Protein(g/l)	36	6.117	0.157		0.993
Albumin(g/l)	36	2.817	-0.085		0.977
Globulin(g/l)	36	3.217	0.258		0.966
Glucose(g/l)	36	63.563	0.342		0.957
Cholesterol(g/l)	36	59.667	-1.650		0.987
Creatinine(g/l)	36	65.583	-1.567		0.964
QUADRATIC MODEL					
Total Protein(g/l)	36	5.617	0.657	-0.100	0.993
Albumin(g/l)	36	2.504	0.228	-0.063	0.977
Globulin(g/l)	36	3.029	0.446	-0.038	0.966
Glucose(g/l)	36	64.067	0.341	0.124	0.967
Cholesterol(g/l)	36	66.125	-8.108	1.292	0.988
Creatinine(g/l)	36	70.583	-6.567	1.000	0.964

Parameters	N	A	B	C	R^2
ALLOMETRIC MODEL					
Total Protein(g/l)	36	6.221	0.056		0.933
Albumin(g/l)	36	2.718	-0.054		0.976
Globulin(g/l)	36	3.428	0.146		0.965
Glucose(g/l)	36	64.358	0.450		0.971
Cholesterol(g/l)	36	58.696	-0.070		0.988
Creatinine(g/l)	36	64.744	-0.062		0.964

values for the linear, quadratic and allometric models followed the same trends. In the linear model, the regression coefficient (b) showed that every 25mg/kg BW drenching of AMSE resulted in 0.157 g/l change in total protein, -0.085 g/l in *Albumin*, 0.025 g/l in *Globulin*, 0.342 g/l in *Glucose*, -1.650 g/l in *Cholesterol*, and -1.567 g/l in creatinine. In the quadratic regression model, the c values indicated that the direction of change was downward (negative) for total protein, *Albumin* and *Globulin* while the direction of change was upward (positive) for *Glucose*, *Cholesterol* and creatinine. In the allometric regression model, exponential changes of 0.056 (total protein), -0.054 (*Albumin*), 0.146 (*Globulin*), 0.450 (*Glucose*), -0.070 (*Cholesterol*) and -0.062 (creatinine) were observed. The coefficient of determination (R^2) values were very high indicating that the models were reliable in predicting the changes in the serum biochemical parameters of WAD bucks drenched with AMSE.

Discussion

Aframomun melegueta has been reported by Chiejina and Ukeh (2012) to be a vital constituent in traditional medicine for the treatment of many infections. Mohammed *et al*., 2013 reported that *Aframomun melegueta* contains high levels of phytochemicals such as alkaloids, flavonoids, cardiac glycosides, terpene, steroids, saponins and resins except tannins and balsams were not detected. The high phytochemical content in the A. melegueta seed extract is the reason for its usage medically. No significant effects (P>0.05) were observed in all the haematological parameters measured except in the red blood cells. This indicates that the A. melegueta seed extract does not influence the haematological parameters. The significant reduction in the value of the red blood cells could be attributed to the decrease in the value of the total protein. No significant effects (P>0.05) were observed in all the serum biochemical parameters measured. There was a significant effect (P>0.05) of the A. melegueta seed extract on all blood fatty acid constituents of the West African Dwarf bucks included in this study. Tijani and Luka, 2013 reported that there were no significant effects observed in the Total protein and *Albumin* of rats fed A. melegueta in all treated groups. Total serum protein and *Albumin* are *genera*lly influenced by total protein intake (Onifade and Tewe, 1993). , . No significant effect was observed on the haematological parameters analysed in this study. *Cholesterol*s are essential components of cell membranes including the white blood cells. They are needed for their shapes and specific functions. An increase in WBC levels may raise suspicion of infection or contamination of feed during administration.

There was a significant reduction in the serum biochemical parameters analysed in this study. Denke *et al*., (2006) reported that several hypotheses have been advanced for the serum total *Cholesterol* lowering effect. The stimulation of the oxidation of *Cholesterol* to bile acids as it is also in the case of polyunsaturated fatty acids. The results from this study are in compliance with the observations of Denke *et al*., (2006) that the fact that high lipid diets low in micronutrients and refined dietary sugars lack minerals and vitamins and are often called ´empty calories´ because they draw upon the

body nutrients to be metabolised into the system and when these nutrients are depleted, the metabolism of *Cholesterol* and fatty acids is impeded, contributing to higher *Cholesterol* levels and promoting obesity due to higher levels of fatty acids on the organs and tissues

The reduction in the serum biochemical parameters investigated in this study is contrary to the result obtained from the study of Nwaehujor *et al*., 2014 who investigated the hepatotoxicity of methanol seed extract of *Aframomum melegueta* in Sprague-Dawley rats although the increase was observed in the rats administered a dose of 300 mg/kg. The high dosage couldbe a contributing factor.

The studies conducted by Tijani and Luka, (2013) and Akpanabiatu *et al*., (2013) reported a significant reduction in the values of the blood fatty acid constituents of rats fed with *Aframomun melegueta* which is in agreement with the results obtained from this study on the values of the blood fatty acids.

Conclusion

The study concluded that the haematological, blood fatty acid and serum biochemical constituents of West African dwarf bucks drenched with *Aframomum melegueta* extracts were not significantly affected after three hours post-treatment. Therefore, the effect of *Aframomum melegueta* extracts is not instantaneous. Hence, more study should be carried out to evaluate the haematological, blood fatty acid and serum biochemical constituents of West African dwarf bucks drenched with *Aframomum melegueta* extracts over a longer post-treatment duration.

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PERFORMANCE AND HAEMATOLOGICAL PARAMETERS OF WEST AFRICAN DWARF GOATS FED WATER HYACINTH ENSILED WITH BREADFRUIT

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Abstract

Ruminant animal production in the dry season is usually problematic as weight losses are experienced arising from scarcity of forages. Conservation of water hyacinth (WH) (*Eicchornia crassipes*) ensiled with breadfruit (BF) can help to bridge this gap. Water hyacinth was ensiled for 28 days with different proportions (0%, 10%, 20%, 30%, and 40%) of BF as feed for West African dwarf (WAD) goats and were designated as diets 1, 2, 3, 4 and 5 respectively. Twenty five (25) WAD goats of both sexes weighing between 4.24 - 4.50kg were randomly allotted to the experimental diets. Data on the proximate composition, growth, nutrient utilization and haematology were assessed and analyzed.

Increasing proportion of BF in silage diets greatly improved the proximate composition, except for crude protein and ash. Feed intake was significantly affected by increasing levels of BF in diets. Average daily gain (g/d) for goats were similar in diets 3 (36.36 g/d), 4 (39.24 g/d), and 5 (37.93 g/d) and higher ($P<0.05$) than those for animals on diet 1 (29.48 g/d). The feed conversion ratios for goats on diets 1 and 2 (9.65 and 9.53 respectively) were poorer than those obtained for goats fed diets 3, 4 and 5 (7.86, 7.55 and 7.81 g/d), respectively. Haematological parameters improved while *Glucose* values (54.76-86.31mg/dl) increased with increasing proportion of BF in the silage diets.

Water hyacinth ensiled with breadfruit has potential as a feed for ruminants with optimum results in diets with breadfruit inclusion levels of 30%.

Keywords: Breadfruit, Feed Intake, Goats, Growth, Silage, Water hyacinth.

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Introduction

It is increasingly important to devise strategies for ensuring continuous accessibility to quality feedstuff by ruminant animals all year round. Inadequate supply of quality forage on a year round basis and the high cost of conventional feedstuffs are major problems to the productivity of ruminants in Nigeria (Olorunnisomo, 2008). In many tropical countries, the major feed resources upon which cattle and other ruminants live come from grazing mainly on poor quality annual and perennial grasses from natural pastures. During the dry seasons, grasses are scarce and in some instances are not available, and where available, are not adequate to meet the animals' requirements for growth, maintenance and production (Adegbola, 1985). The resultant effect of this is the on and off (staircase) growth rate pattern exhibited by the animals over time, which consequently negatively affects the overall productivity of the animals (Babayemi and Bamikole, 2006a; Ibhaze and Fajemisin, 2015). Concerted efforts in research has been directed towards mitigating these effects and creating awareness to improve and supplement grasses, especially in the dry season, with crop residues and agro-industrial by-products as well as the use of legume and browse plants. However, these interventions are also affected by costs, seasonality and availability.

The conservation of forages is a step towards achieving sufficiency and sustainability in ruminant production. One of the conservation methods is the production of silage. Silage production is a form of forage conservation where a forage, crop residue or agricultural by-product is preserved in its near fresh form for later use during the off season period, by acids either artificially added or produced by natural preservation, in the absence of air (Moran, 2005). Silage making is an important tool for farmers in the preservation of surplus feed during the wet season in order to ensure all year round availability of feed (Ibhaze *et al*., 2015). Many researchers have worked on the production of silages with different forages. One of these forages is *Eichhornia crassipes* (Water hyacinth) which is a rigorous, free floating, fresh water weed of the family Pontederiaceae. This forage's nutritive value is well documented (Akinwande *et al* 2011). However, there is dearth of information on its utilization as silage in a conserved form. Inclusion of silage additives helps to improve silage quality and animal performances. Molasses, sugar beet, bagasse and most recently sugar cane have been used as fermentation stimulants. Wheat offal, poultry litter, citrus pulp and cassava peels have also been documented as additives. However, cost and availability are often a limiting factor.

Breadfruit (*Artocarpus artillis*) contains easily fermentable carbohydrates in the form of sugars in its matured state and is available in excess of requirement because of its underutilization. The excess constitutes a waste beneath trees during its season. Furthermore, literature is scanty on the utilization of breadfruit as an additive in silage production. Meanwhile, the environmental impacts of water hyacinth and breadfruit remains a cause for concern.

The objective of this study was to assess the nutrient digestibility, growth performance and haematological parameters of West African Dwarf goats fed water hyacinth ensiled with varying levels of breadfruit.

Materials and Methods

Experimental station and duration

The experiment was carried out at the Sheep and Goat Unit, Obafemi Awolowo University Teaching and Research Farm, Ile – Ife located approximately between latitude 70 31°N and 70 33°N; and longitudes 40 33°E and 40 34°E (Amujoyegbe *et al*., 2008). The experiment lasted sixteen (16) weeks.

Silage production and experimental diets

Water hyacinth (WH) was collected from Itoikin River, Epe road Lagos State. Breadfruit (BF) and sawdust (SD) were sourced within Ile-Ife town in Osun State, Nigeria. Roots of harvested water hyacinth were discarded while the vegetative parts were wilted and used for making silage. These

were chopped into smaller pieces of about 2cm – 3cm to aid compaction and mixed with chopped breadfruits at varying inclusion levels of 0%, 10%, 20%, 30% and 40% of the total silage diet. Sawdust was included at a constant level of 10%. The WH: BF: SD mixture was packed, compacted and sealed in thick polythene bags to create anaerobic conditions for proper fermentation. The silage was ensiled for 28 days after which the bags were opened. Silage diets (constituting of 60% of the total diet) were fed at 8:00hrs while a compounded concentrate diet (the remaining 40%) was fed at 15:00hrs.

Experimental animals and their management

A total of twenty-five growing WAD goats of both sexes aged 5 - 7 months and weighing 4.50 ± 0.34 kg were randomly assigned to five experimental diets in a completely randomized design. There were 5 goats per treatment with each animal serving as a replicate. The goats were housed in an open sided, well-lighted and adequately ventilated building with a slated floor. The house was disinfected before the animals arrived. The animals were vaccinated against Pestes des Petits Ruminants, quarantined and observed for any disease symptoms for seven days. The goats were also dewormed and treated against ectoparasites using ivomec ®. Prior to the commencement and throughout the experiment, the animals were feed at 5% of their body weight. Water was supplied *ad libitum*.

Digestibility, nitrogen balance and growth studies

A digestibility trial was carried out for two weeks preceded by a 14-days' adaptation period with three goats randomly selected per treatment and moved into metabolism cages with facilities for the separate collection of faeces and urine. The total faeces voided per animal was weighed and aliquot samples taken per day and dried in the oven at 70°C for 24 hours for dry matter determination. The daily stored samples of faeces for each animal were bulked, thoroughly mixed, ground and sub-sampled for chemical analysis. The volume of urine produced by each animal was measured daily. 10% of the daily urine produced was taken and the volatilization of nitrogen from the urine was prevented by introducing 0.1N of HCl into the urine which was then stored in a deep freezer and later analyzed for nitrogen determination. The growth trial lasted for twelve weeks. Each animal was weighed using a hanging scale and weighing sack before the commencement of the study and subsequently at weekly intervals throughout the experimental period. The parameters measured included; feed refusal, feed intake and weight gain.

Blood samples collection and analysis

Blood samples were collected from three animals per treatment via the jugular vein of the animals a week before the commencement of the experiment and on the 12th week of the feeding trial. Prior to feeding in the morning, the animals were bled to assess their blood profiles. About 5ml of blood was obtained from each animal with 2.5ml collected in a plain bottle and the other 2.5 ml in EDTA containing bottles. Centrifugation of the blood collected in plain bottles was carried out according to the methods of Mitruka and Rawnsley (1977) to obtain sera. The serum metabolites (*Glucose*, total protein, *Albumin*, urea, *bilirubin*, *calcium*, *phosphorus* and creatinine) were determined according to the Randox procedure of chemical analysis (2010). The readings were carried out using a photo spectrometer in the laboratory and *Globulin* values were estimated.

Statistical analysis

Data obtained for each parameter was subjected to a one way analysis of variance using the *General* Linear Model Procedures of SAS (2001) while differences between means were separated using the Duncan's Multiple Range Test of the same package.

Results

Table 1 shows the gross composition (%) of silage diets while Table 2 shows the gross composition of the concentrate diet. The calculated crude protein, crude fibre and metabolizable energy were 13.45%, 12.03% and

1447.60 Kcal/kg respectively.

Table 3 shows the chemical composition (g/100g) of experimental diets. The dry matter values ranged from 14.21 to 28.44 g/100g. Diet 1 had the lowest value (14.21 g/100g) while diets 3 and 4 (28.03 and 28.44 g/100g) were similar but higher (P>0.05) than others. The ash content was highest (P> 0.05) in diet 1 and progressively reduced as the breadfruit content increased across the diets. The ether extract increased with increasing levels of breadfruit in the diets and the highest ether extract values were observed in diet 5 (2.70g/100g). The crude protein values ranged from 12.03% (diet 1) to 9.18% (diet 5). The acid detergent fibre content reduced as breadfruit increased in the diets. The cellulose content of diets also followed the same trend as the ADF content.

Apparent nutrient and energy digestibility of WAD goats fed experimental diets are presented in Table 4. The dry matter digestibility values for diets 2, 3, 4 and 5 were similar but higher (P<0.05) than values for diet 1 (67.61%). DDMI values for diets 4 and 5 were significantly (P<0.05) higher than the values for diet 1. The crude protein and crude fibre intake digestibilities for diet 1 (86.87 and 81.70% respectively) were significantly (P<0.05) higher than for other diets. Ash digestibility, (60.30%) for diet 5 was significantly (P<0.05) higher than digestibilities of other diets by goats. The Neutral detergent fibre digestibilities for diets 4 and 1 (66.47 and 63.86 %) were not significantly (P>0.05) different but higher (P<0.05) than diets 2, 3 and 5. Diets 1, 4 and 5 had significantly (P<0.05) higher acid detergent fibre digestibilities (76.06, 74.17 and 73.81%) than diets 2 and 3 (63.39 and 62.13%). The digestibility of acid detergent lignin by goats was significantly (P<0.05) higher in diet 1 (43.88%) than values (26.68, 27.35, 29.33 and 37.35%) obtained for goats on diet 2, 3, 4, and 5 respectively.

Table 5 shows the growth performance characteristics of WAD goats fed experimental diets. Total average daily feed intake (ADFI) increased (P>0.05) across the diets with an increase in the breadfruit content. The average final live weight gain ranged from 6.90 kg (diet 1) to 7.60 kg (diet 5). The total weight gains were similar in animals fed diets 3, 4 and 5 and higher than those fed diets 1 and 2. The average daily gain was significantly (P<0.05) affected by silage

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

Ingredients/diets	**1**	**2**	**3**	**4**	**5**
Water hyacinth	90	80	70	60	50
Breadfruit	0	10	20	30	40
Sawdust	10	10	10	10	10
Total	**100**	**100**	**100**	**100**	**100**

Table 2: Gross composition of the concentrate diet

Ingredients	**Proportion (%)**
Maize bran	40.0
PKC	24.5
Wheat offal	35.0
Salt	0.25
Premix	0.25
Calculated values	
Crude protein (%)	13.45
Crude fibre (%)	12.03
Metabolizable energy (Kcal/Kg)	1447.60

Table 3: Chemical composition of experimental diets

Parameters/diets (g/100g)	1	2	3	4	5	SEM	Prob.
Dry matter	14.21^{d}	15.99^{c}	28.03^{a}	28.44^{a}	26.64^{b}	0.55	<0.0001
Ash	14.75^{a}	13.80^{ab}	12.86^{bc}	11.72^{c}	9.64^{d}	0.78	0.0003
Ether Extract	1.67^{ab}	1.43^{b}	2.42^{ab}	2.53^{a}	2.70^{a}	0.30	0.0499
Crude fibre	21.48^{c}	30.00^{a}	23.21^{bc}	25.25^{b}	15.23^{d}	1.30	<0.0001
Crude protein	12.03^{a}	10.50^{b}	10.72^{b}	10.28^{b}	9.18^{c}	0.26	0.0008
Nitrogen free extract	50.07^{b}	44.27^{c}	50.79^{b}	50.22^{b}	63.25^{a}	2.68	<0.0001
Fibre fractions							
Neutral Detergent fibre	50.99^{b}	57.48^{a}	49.14^{bc}	47.03^{d}	43.51^{d}	0.69	<0.0001
Acid Detergent fibre	33.16^{b}	38.71^{a}	32.30^{b}	30.74^{b}	23.06^{c}	0.81	<0.0001
Hemicellulose	17.84^{bc}	18.77^{b}	16.84^{cd}	16.29^{d}	20.45^{a}	0.34	<0.0001
Cellulose	30.05^{b}	35.02^{a}	28.90^{b}	27.58^{a}	20.24^{c}	0.77	<0.0001
Acid detergent lignin	3.11^{c}	3.69^{a}	3.40^{b}	3.16^{c}	2.82^{d}	0.05	<0.0001

^{abcd}: means within each row with different superscript are significantly different ($p<0.05$)
1= 0%BF + 90%WH + 10% SD; 2 = 10%BF + 80%WH + 10%SD; 3 = 20%BF + 70%WH + 10%SD; 4 = 30%BF + 60%WH + 10%SD. BF = Breadfruit; WH = Water Hyacinth; SD = Sawdust; SEM = Standard Error of Mean; Prob. = Probability level.

Table 4: Apparent nutrient digestibility of experimental diets by WAD goats

Parameters (%) / Diets	1	2	3	4	5	SEM	Prob.
DM	67.61^{b}	74.67^{ab}	73.21^{ab}	76.21^{a}	80.78^{a}	1.44	0.0294
CP	62.74^{d}	73.47^{bc}	65.42^{cd}	86.86^{a}	79.51^{ab}	2.62	0.0008
CF	81.70^{a}	72.52^{b}	74.67^{ab}	69.06^{b}	74.68^{ab}	1.75	0.0264
EE	75.13	73.74	77.42	70.31	81.86	1.38	0.3268
ASH	52.82^{b}	41.14^{c}	50.43^{b}	42.70^{c}	60.30^{a}	2.06	<.0001
NFE	79.53^{a}	70.86^{ab}	75.50^{a}	61.66^{b}	79.53^{a}	1.34	0.0266
Fibre fraction							
NDF	63.86^{a}	60.82^{b}	54.26^{c}	66.47^{a}	58.66^{b}	0.87	<0.0001
ADF	76.06^{a}	63.39^{b}	62.13^{b}	74.17^{a}	73.81^{a}	1.81	<0.0001
HEMICELLULOSE	55.31^{a}	41.18^{b}	32.79^{c}	52.53^{a}	53.72^{a}	1.46	<0.0001
CELLULOSE	73.46^{a}	75.46^{a}	77.79^{a}	76.99^{a}	66.34^{b}	1.64	<0.0001
ADL	43.88^{a}	26.68^{c}	27.35^{c}	29.33^{c}	37.35^{b}	1.21	<0.0001

$^{a, b, c, d}$: Means within each row with different superscript are significantly different ($p< 0.05$)
1 = 0%BF + 90%WH+ 10%SD; 2 = 10%BF + 80%WH+ 10%SD; 3 = 20%BF + 70%WH+ 10%SD; 4 = 30%BF + 60%WH+ 10%SD; 5 = 40%BF + 50%WH+ 10%SD ; SD = Sawdust; BF = Breadfruit; WH = Water hyacinth; SEM = Standard error of mean; Prob = Probability level; DM: Digestibility of dry matter intake; CP: Digestibility of crude protein intake; CF: Digestibility of crude fibre intake; EE: Digestibility of ether extracts intake; ASH: Digestibility of ash; NFE: Digestibility of nitrogen free extract; NDF: Digestibility of Neutral detergent fibre ADF: Digestibility of Acid detergent fibre; HEMICELLULOSE: Digestibility of Hemicellulose; CELLULOSE: Digestibility of cellulose; ADL: Digestibility of Acid detergent lignin.

Table 5: Growth performance characteristics of WAD goats fed experimental diet

Parameters (%) / Diets	1	2	3	4	5	SEM	Prob.
ADFI (g/day)							
Concentrate	145.90^{a}	146.40^{a}	146.79^{a}	139.33^{c}	142.30^{b}	0.92	<0.0001
Silage	135.40^{c}	137.70^{bc}	138.92^{b}	148.50^{a}	149.65^{a}	0.86	<0.0001
Total ADFI	281.30^{c}	284.10^{bc}	285.71^{bc}	287.83^{ab}	291.95^{a}	1.60	0.0019
AILW	4.24	4.53	4.45	4.50	4.41	0.14	0.9603
AFLW	6.90^{b}	7.08^{b}	7.50^{a}	7.80^{a}	7.60^{a}	0.11	<0.0001
TWG (kg)	2.47^{c}	2.54^{bc}	3.05^{ab}	3.30^{a}	3.19^{a}	0.17	0.0079
ADG (g/day)	29.48^{c}	30.29^{bc}	36.36^{ab}	39.24^{a}	37.93^{a}	2.07	0.0080
FCR	9.65^{a}	9.53^{a}	7.86^{b}	7.55^{b}	7.81^{b}	0.54	0.0230

$^{a, b, c, d}$: Means within each row with different superscript are significantly different (p< 0.05)
1 = 0%BF + 90%WH+ 10%SD; 2 = 10%BF + 80%WH+ 10%SD; 3 = 20%BF + 70%WH+ 10%SD; 4 = 30%BF + 60%WH+ 10%SD; 5 = 40%BF + 50%WH+ 10%SD ; SD = Sawdust; BF = Breadfruit; WH = Water hyacinth; SEM (±): Standard error of mean; PROB: Probability level; ADFI: Average daily feed intake; AILW: Average initial live weight; AFLW: Average final live weight; TWG: Total weight gain; ADG: Average daily gain; Av: Final live weight gain ranged from 6.90 kg (Diet 1) – 7.80 kg (Diet 4); FCR: Feed conversion ratio

Table 6: Nitrogen utilization of WAD goats fed experimental diet

Parameters (%) / Diets	1	2	3	4	5	SEM	Prob.
Nitrogen intake (g/day)	3.39^{a}	2.57^{b}	2.63^{b}	2.62^{b}	2.47^{b}	0.19	0.0409
Faecal nitrogen (g/day)	1.12^{a}	0.33^{b}	0.49^{b}	0.35^{b}	0.48^{b}	0.13	0.0108
Urinary nitrogen (g/day)	0.24^{b}	0.28^{b}	0.27^{b}	0.44^{a}	0.34^{ab}	0.04	0.0215
Nitrogen loss (g/day)	1.35^{a}	0.61^{b}	0.77^{b}	0.79^{b}	0.81^{b}	0.14	0.0326
Nitrogen balance (g/day)	2.04	1.96	1.85	1.82	1.65	0.19	0.6570
Nitrogen retention (%)	60.02^{b}	76.75^{a}	70.09^{ab}	69.49^{ab}	67.28^{ab}	4.57	0.2186

$^{a, b, c, d}$: Means within each row with different superscript are significantly different (p< 0.05)
1 = 0%BF + 90%WH+ 10%SD; 2 = 10%BF + 80%WH+ 10%SD; 3 = 20%BF + 70%WH+ 10%SD; 4 = 30%BF + 60%WH+ 10%SD; 5 = 40%BF + 50%WH+ 10%SD; SD: Sawdust; BF: Breadfruit; WH: Water hyacinth; SEM: Standard error of mean; PROB: Probability level

diets. The feed conversion ratios for goats on diets 1 and 2 (9.65 and 9.53 respectively) were significantly (P<0.05) higher than the values of 7.86, 7.55 and 7.81 obtained for goats fed on diets 3, 4 and 5 respectively.

Nitrogen utilization of WAD goats fed experimental diets are shown in Table 6. Nitrogen intake, *Faecal* nitrogen and nitrogen loss (g/day) were significantly higher (P<0.05) in goats fed diet 1 than other diets. There was no significant difference (P>0.05) in nitrogen intake, *Faecal* nitrogen and nitrogen loss values for goats fed diets 2, 3, 4 and 5. Urinary nitrogen was significantly higher in goats fed diet 4 (0.44 g/day) than for those fed diets 1, 2 and 3 (0.24, 0.28, 0.27 g/day respectively). The nitrogen balance in goats was not affected by silage diets. Nitrogen retention (%) was significantly (P<0.05) higher in diet 2 (76.75%) than diet 1 (60.02%).

Table 7 shows the haematological profiles of WAD goats fed experimental diets. Values obtained for White blood cell counts, Mean corpuscular *Haemoglobin* (Hb) concentrations, *Lymphocytes*, *Monocytes* and *Basophil*s were not significantly (P>0.05) affected by diets. Silage diets significantly (P<0.05) increased the Packed Cell Volume (PCV) and Hb concentration. The *Haemoglobin* concentration values (8.00 and 7.78g/dl) for goats fed diets 3 and 5 were significantly (P<0.05) higher than those observed (6.34,

7.11 and 7.39g/dl) for goats fed silage diets 1, 2 and 4 respectively. The packed cell volume increased from diet 1 to 5, but was significantly ($P<0.05$) different between animals fed diet 1 and other diets. The mean corpuscular volume (MCV) differed significantly across the diets and ranged between 36.85 and 50.09fL

The serum biochemical indices are presented in Table 8. The total protein, *Albumin*, urea and creatinine were unaffected by the silage diets. *Glucose* values ranged from 54.76 – 86.31 mg/dl and increased as the breadfruit content increased in the diets. An increase in breadfruit in the diets resulted in a reduction in *bilirubin*, *calcium* and *phosphorus* values.

Table 7: Haematological profile of WAD goats fed Experimental diets

Parameters (%) / Diets	**1**	**2**	**3**	**4**	**5**	**SEM**	**Prob.**
Hb (g/dl) Initial	11.16	11.19	10.53	10.54	10.52	0.81	0.9384
Hb (g/dl) Final	6.34^{b}	7.11^{ab}	8.00^{a}	7.39^{ab}	7.78^{a}	0.34	0.0437
RBC (×1012/l) Initial	10.67	10.42	10.17	10.17	9.81	0.67	0.9167
RBC (× 1012/l) Final	6.34^{ab}	5.92^{b}	6.04^{b}	6.48^{a}	6.32^{ab}	0.14	0.0433
PCV (%)	19.67^{b}	22.66^{a}	24.33^{a}	24.00^{a}	24.00^{a}	0.89	0.0218
WBC (× 109/l)	6.70	6.52	6.45	6.57	6.88	0.17	0.4732
MCV (fL)	36.85^{b}	46.40^{ab}	50.09^{a}	43.90^{a}	45.11^{ab}	3.73	0.0280
MCHC (g/dl)	32.18	31.31	32.99	30.78	32.27	1.93	0.9325
Lymphocytes (%)	69.33	68.00	69.33	69.30	67.67	0.78	0.4053
Neutrophils (%)	28.00^{bc}	31.67^{a}	26.00^{c}	27.67^{a}	30.00^{ab}	0.98	0.0185
Eosinophil (%)	1.00^{b}	1.67^{ab}	2.00^{a}	1.33^{b}	1.33^{b}	0.44	0.5962
Monocytes (%)	2.00	1.67	1.33	1.67	1.67	0.47	0.6169
Basophils (%)	0.08	0.08	0.07	0.06	0.03	0.04	0.8759

$^{a, b, c, d}$: Means within each row with different superscript are significantly different ($p< 0.05$)
1 = 0%BF + 90%WH+ 10%SD; 2 = 10%BF + 80%WH+ 10%SD; 3 = 20%BF + 70%WH+ 10%SD; 4 = 30%BF + 60%WH+ 10%SD; 5 = 40%BF + 50%WH+ 10%SD ; SD = Sawdust; BF = Breadfruit; WH = Water hyacinth; SEM (±): Standard error of mean; PROB: Probability level; Hb: *Haemoglobin*; PCV: Packed Cell Volume; RBC: Red Blood Cell; WBC: white Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular *Haemoglobin*; MCHC: Mean Corpuscular *Haemoglobin* Concentration

Table 8: Serum biochemical response of WAD Goats fed Experimental Diets

Parameter(s)	**1**	**2**	**3**	**4**	**5**	**SEM**	**Prob.**
Glucose (mg/dl)	54.76^{c}	61.48^{b}	62.93^{b}	63.26^{b}	86.31^{a}	2.10	<0.0001
Total Protein (g/dl)	10.00	11.14	10.00	11.97	10.31	0.72	0.2932
Albumin (g/dl)	4.55	4.57	4.12	3.72	4.30	0.30	0.3219
Globulin (g/dl)	5.45^{b}	6.58^{ab}	5.88^{ab}	8.28^{a}	6.01^{ab}	0.72	<0.0001
Bilirubin (mg/dl)	2.02^{a}	1.98^{a}	1.97^{a}	1.11^{b}	1.19^{b}	0.05	0.0407
Urea (mg/dl)	17.56	18.47	18.01	18.36	17.90	1.06	0.9718
Calcium (mg/dl)	10.31^{a}	11.68^{a}	9.94^{a}	6.94^{b}	7.13^{b}	0.58	0.0006
Phosphorous (mg/dl)	15.02^{a}	13.25^{ab}	11.47^{bc}	11.54^{bc}	8.89^{c}	0.89	0.0076
Creatinine (mg/dl)	0.87	0.85	1.02	0.74	0.77	0.14	0.6562

$^{a, b, c, d}$: Means within each row with different superscript are significantly different ($P< 0.05$)
1 = 0%BF + 90%WH+ 10%SD; 2 = 10%BF + 80%WH+ 10%SD; 3 = 20%BF + 70%WH+ 10%SD; 4 = 30%BF + 60%WH+ 10%SD; 5 = 40%BF + 50%WH+ 10%SD SD = Sawdust; BF = Breadfruit; WH = Water hyacinth; SEM (±): Standard error of mean; PROB: Probability level

Discussion

The increase in dry matter with increasing inclusion levels of breadfruit may be attributed to the relatively high dry matter of breadfruit (73.4g/100g) and the low dry matter content of water hyacinth. The dry matter values obtained were similar to values of 24.2 - 33.4% obtained by Tham *et al*., (2012) for fresh water hyacinth and also similar to values of 29.95- 38.92% reported for ensiled and unensiled 4, 6 and 8 weeks regrowth vetiver grass (Falola *et al*., 2013). A dry matter value of 28.44% obtained in diet 4 is similar to the value of 25.35% obtained by Aboud *et al*., (2005) who ensiled water hyacinth with molasses at a 20% inclusion level. The high ash value obtained in this study may be attributed to the high rate at which effluents flow into the water body and the water hyacinth's ability to mop up excess nutrients from its surrounding water body. The high ash content of Water hyacinth indicates that the plant will be a good source of minerals. Crude protein (CP) levels of silage diets obtained in this study were lower than the values of 12 – 16 % reported by Reza and Khan (1981) for water hyacinth fed to cattle. These differences may be due to geographical differences, human activities around the water body in which the plants thrive and the stage of maturity of the plants when fed. The reduction in CP levels of diets as breadfruit inclusion increased may be attributed to the low crude protein content of breadfruit, However the CP content of the diets meets the protein requirement for ruminants which is 8g /100 g DM (NRC 1981). High nitrogen free extract (NFE) in diet 5 may be attributed to the high inclusion level of breadfruit which in turn increased the carbohydrate fraction in terms of NFE.

The Dry Matter (DM) digestibility of fresh or dried water hyacinth is usually low, ranging between 47-58% (Abdelhamid and Gabr, 1991; Hira *et al*., 2002). However Silage made from water hyacinth is more digestible, with DM digestibility values of 67 and 64% obtained in water buffalo and sheep respectively (El-Serafy *et al*., 1980). However, the dry matter digestibility (67.61% - 80.78%) obtained in this study was higher than those reported for buffalo and sheep (El-Serafy *et al*., 1980) and for sheep fed ensiled water hyacinth as a replacement for para grass (Nguyen, 2016) but similar to values of 67.35-55.91% reported for WAD goats fed agro industrial by-products and Pennisetum purpureum hay as dry season feed (Obe and Yusuf, 2017). The higher DM digestibility in diets with increased additive could be as a result of the fermentation process due to the fermentable carbohydrates from the breadfruit additive. This corroborates the findings of Bereenok *et al*., (2012) who reported that the use of molasses as a silage additive was associated with a significantly higher DM digestibility compared to the silage without additive. The high protein digestibility also observed agrees with the earlier observation of Sayed (2009) who reported that an increase in the dietary protein intake level may cause changes in the process of rumen fermentation and allow more protein digestibility. It also agrees with the results of Geerts *et al*. (2004), who found that the nutrient digestibility of diets increased with increasing crude protein content. This observation agrees with the conclusion of Arigbede *et al*., (2005) and Fasae *et al*., (2005), that protein supplementation enhanced digestibility. Furthermore, the crude fibre digestibility values (69.06 - 81.73%) in this study were similar to the values (68.04 – 76.14%) reported by Ahamefule and Elendu, (2010) who fed cassava leaf-maize offal based diets to WAD bucks. Differences observed in the fibre digestibility of diets by animals may be as a result of variation in the NDF and ADF contents of the experimental diet based on the increasing levels of breadfruit and corresponding decrease in the water hyacinth across the silage diets. These results agree with the observation of Norton, (1994) who reported that the fibre fraction of food has the greatest influence on digestibility. The digestibility of Neutral detergent fibre (54.26% - 66. 47%) obtained in this study were similar to values (65.6 – 66.7%) reported by Nguyen (2016) for water hyacinth silages fed to sheep.

Nitrogen balance is described as a

good indicator of the protein value of a diet (Babayemi and Bamikole, 2006). All the diets in this study had positive nitrogen balances, which indicated adequacy in the protein requirement for maintenance. The nitrogen balances obtained in this study were higher than the 1.34 – 1.69 g/day obtained by Babayemi and Bamikole, (2006) when Tephrosia bracteolata was fed to WAD goats. It was however lower than the values of 2.75 – 3.75 reported by Oni *et al*., (2010). Nitrogen retention is the proportion of nitrogen utilized by farm animals from the total nitrogen intake for body processes, hence the more the nitrogen consumed and digested, the more the nitrogen retained and vice versa. This trend was also observed by Okeniyi *et al*. (2010).

Reports by Ajayi *et al*. (2005) and Ososanya (2010) indicated that feed intake is an important factor in the utilization of feed by livestock. The total average daily feed intake of silage diets by goats in this study increased as the inclusion levels of breadfruit increased. This observation agrees with the report of Yousuf and Adeloye (2010) who observed that intake of feeds by goats depend on palatability and fibre content of the diets. The use of breadfruit as a source of fermentable carbohydrate in the silage diets improved the palatability and subsequent intake. Baldwin *et al*., (1975) reported that using very palatable materials in ensiling increased the acceptability of diets by animals. The animals in all the treatments maintained a positive weight gain. The daily weight gains in this experiment were good when compared with the results of Ajayi *et al*. (2005) who obtained 23.81 – 46.64 g/day for West African Dwarf goats fed Mango (Mangifera indica), Ficus (Ficus thonningii), Gliricidia (Gliricidia sepium) foliages and concentrates as supplements to a basal diet of guinea grass (Panicum maximum). The Average daily gains (29.48 – 39.24 g/day) obtained in this study were higher than values (12.2 – 25.6g/day) obtained by Mako, (2013) who fed sun cured water hyacinth replacing guinea grass by up to 90%. Similarly, the results of the FCR revealed that the WAD goats fed 20%, 30% and 40% inclusion levels of breadfruit utilized the diets for body weight gain better than those fed 0% and 10% breadfruit inclusion levels. The positive response obtained for average daily weight gain and feed conversion ratio in goats fed silage diets with 20%, 30% and 40% inclusion levels of breadfruit could be probably used to further attest to the superiority of goats on those diets in terms of nutrient utilization for body weight gain over animals fed diets 1 and 2.

The *Haemoglobin* content (10.52 – 11.19g/dl) obtained in this study before the experiments were higher than the values obtained at the end of the experiment (6.34 – 8.00g/dl). Values obtained before the experiment were in the range of values (7 – 15g/dl) reported by Daramola *et al*. (2005). The final value of 6.34g/dl obtained for goats fed WH silage without breadfruit was lower than the normal physiological ranges reported by Daramola *et al*. (2005) for WAD goats. This may be attributed to the haemolytic effect of WH silage on WAD goats. Except for diet 1, the values for packed cell volume (PCV) obtained for goats in this study were within the range (21 – 35%) reported by Daramola *et al*., 2005. These values were however lower than values (28.80 – 35.60%) reported by Ayandiran *et al*., (2012) and (25.86 - 32.40%) reported by Goska *et al*., (2017) for Bunaji bulls fattened on varying inclusions of groundnut haulms and maize offal. The red blood cell (RBC) counts obtained after the feeding trial was lower than the normal physiological range (7.5 - 15.0 x 1012/L) which also indicated the haemolytic effect of the silage diets on WAD goats. The reduction in red blood cells in goats fed silage diets indicates that the oxygen carrying capacity of red blood cells was compromised and may have caused anaemia (Okwori *et al*., 2016). The white blood cell (WBC) counts obtained in this study were within the normal physiological range (4.0 – 12.0 x 109/L) reported by Jain (1993). The RBC, Mean corpuscular volume (MCV), Mean corpuscular *Haemoglobin* (MCH) and Mean corpuscular *Haemoglobin* concentrations were within the ranges reported by Daramola *et al*., 2005 for WAD goats. Also the WBC differential counts obtained for goats in this study were within the range reported by Jain,

(1993). Factors influencing the haematological parameters of WAD goats are: environmental condition (Vecerek, *et al*., (2002); dietary content (Odunsi *et al*., 1999); fasting (Lamošová *et al*., 2004), age (Seiser *et al*., 2000), administration of drugs (Khan, *et al*., 1994), anti-aflatoxin treatment (Oguz *et al*., 2000) and continuous supplementation of vitamins (Tras *et al*., 2000)

The blood *Glucose* levels (54.76 – 86.31mg/dl) of animals obtained in this study were higher than the range of 45 - 60 mg/dl reported by Pampori, (2003) for clinically healthy goats. However there were significant ($P<0.05$) reductions in the blood *Glucose* after the feeding trial for diets 1 to 4, although it increased with increasing inclusion of breadfruit. this corroborates the findings of Tyagi (2015) who reported the anti-diabetic properties of water hyacinth. The Serum protein values (6.10 – 11.14g/dl) obtained in this study were higher than the range of 6.1 - 8.5 g/dl reported by Daramola *et al*., 2005 for WAD goats but the total protein observed for goats before the feeding trials were within the range of 6.1 - 7.5g/dl reported by Merck (2011). The serum *Albumin* range from this study was similar to the range of 3.90 - 4.55g/dl reported for WAD goats by Yussuf *et al*. (2012) but higher than the range of 2.98 - 3.43 g/dl reported by Okoruwa *et al*. (2014) and Opara *et al*. (2010) respectively. An increase in the serum *Albumin* above normal levels indicates dehydration and impairment in the functions of the liver, kidneys and digestive system while low *Albumin* suggests poor clotting of blood (Robert *et al*., 2000) and reduction in disease fighting ability of the animal body system which could lead to high mortality (Iheukwumere *et al*., 2005). The differences in the serum biochemical parameters of animals in this study may have been caused by nutritional, environmental and hormonal factors Chineke *et al*. (2002). Normal enzyme levels in serum are a reflection of a balance between synthesis and their release, as a result of the different physiological process in the body (Zilva and Pannall, 1984). The Serum urea levels observed for goats in our study (22.09 - 39.19 mg/dl) were similar to those (20.70 - 30.04 g/dl and 37.9 g/dl) reported by Okoruwa and Agbonlahor (2014) and Opara *et al*. (2010) respectively. An average lower serum urea concentration may be an indicator of better protein quality (Eggum, 1970), while a high level of serum urea has been attributed to excessive tissue protein catabolism associated with protein deficiency (Oduye and Adadevoh, 1976). Treatment diets did not appear to significantly ($P<0.05$) influence the creatinine levels in the blood serum of goats. However, the observed increased levels of creatinine after the feeding trial may be attributed to decreased kidney function. This explains the effectiveness of body mass function in goats as reported by Okoruwa *et al*. (2014). The increased *calcium* and *phosphorus* levels for diets 1 and 2 compared to diets 3, 4 and 5 can be attributed to the higher inclusion of water hyacinth in diets 1 and 2 since water hyacinth has been said to be high in mineral content (Mako, 2013).

Conclusion

The results demonstrated that water hyacinth diets ensiled with breadfruit have potentials as feed for ruminants. Diet 4 consisting of 30% BF and 60% WH appears to elicit the best performance in terms of ADWG and FCR. However, some haematological parameters were negatively affected over the period of the experiment hence, diets consisting of WH and BF silage should not be fed for long stretches otherwise, they should be fed with other feed resources that will compensate for Water Hyacinth's haemolytic properties.

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ASSESMENT OF GASTROINTESTINAL PARASITES IN EXTENSIVELY GRAZED CATTLE IN SOUTHWESTERN NIGERIA

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Abstract

Gastro Intestinal Parasites (GIP) are found both in humans and animals and exert a global influence on livestock husbandry. They are responsible for both sub-clinical and clinical infections which can translate into poor livestock productivity. Thus, constant monitoring of the presence of GIP in a developing country like Nigeria is important. A cross sectional study was undertaken to determine the prevalence of GIP in extensively grazed cattle in two States of Southwest Nigeria. *Faecal* samples were collected per rectum from cattle of different breeds, sex, age and body condition score (BCS) from the late dry season to the early rains of 2017. Samples were analyzed using the simple floatation technique with salt solution as the floatation solution to detect the presence of GIP eggs and oocysts. The helminthes' eggs and protozoa oocysts were identified using standard morphological features. Statistical significance taking p-value to be ≤ 0.05 was recorded between the GIP prevalence and location of herds, mixed infection and BCS while there was no significance between GIP prevalence, age and sex. *Faecal* examinations showed that 226 (66.1%) of the 342 cattle screened had GIP infection; single infections occurred in 40.64% (139/342) while mixed infections were recorded in 25.44% (87/342) of the cattle. From this study GI helminthes and protozoan prevalence were 58.48% (200/342) and 40.06% (137/342) respectively. Of the GIP observed from this study, 51.46% (176/342) were nematodes from the *genera* Strongyles, Strongyloides, *Nematodirus*, *Neoscaris* and *Trichuris*; 4.39% (15/342) were trematodes of *genera Fasciola*, *Paramphistomum* and *Schistosoma* and only 2.63% (9/342) were cestodes of *genera Taenia* and *Moniezia*. The only protozoan identified from this study was Eimeria; 40.06% (137/342). The need for farmer's education about GIP is advocated; Government's role in the regular monitoring, screening and control of GIP through public enlightenment is elucidated because some of the GIP identified are of public health importance.

Keywords: Cattle, Extensive, Gastro intestine, Nigeria, Parasite, Prevalence

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Introduction

Cattle are raised mainly to provide meat and milk, hence contributing to the animal protein sources in Nigeria. This sector contributes 3.2% of the nation's Gross Domestic Product (GDP) and is primarily dominated by pastoralists that possess approximately 90% of the cattle herds in the country (Abass, 2012; Koster and de Wolff, 2012). The Gastro Intestinal (GI) tracts of ruminants can harbor different types of parasites which can either cause clinical or sub-clinical infections (Rafiullah *et al*., 2011). GI parasites have been linked to decreased productivity in livestock production (Badran *et al*., 2012), resulting in both direct and indirect economic losses. Despite the major losses attributed to gastro-intestinal parasitism, the impacts are often overlooked as the majority of infected animals show little or no obvious clinical signs. This can be attributed to the fact that the effects of these parasites are gradual and chronic (Raza *et al*., 2010). The prevalence and frequency of occurrence of gastro-intestinal parasites are dependent on environmental factors, like humidity, temperature, rainfall, vegetation and management practices (Teklye, 1991).

Gastrointestinal parasites are ubiquitous with various pathogenic *genera* that are responsible for morbidity and mortality in livestock (Larsson *et al*., 2011; Thumbi *et al*., 2013). GI parasites are a major cause of concern worldwide. However, their impact is more pronounced in developing countries (Regassa *et al*., 2006). This is due to the abuse and misuse of anti-helmintics and anti-protozoan drugs that are used indiscriminately without proper diagnosis, despite the increasing anti-microbial resistance among pathogenic agents. Furthermore, surveillance and periodic monitoring of GI parasites in pastoralists' herds in Nigeria is not functional. Therefore, this study was carried out to determine the prevailing gastro-intestinal parasites among pastoralists' cattle in two States of the Southwest Nigeria, and their associated risk factors. The outcome of this study seeks to provide the basis for enlightenment and to suggest best health practices to manage GIP among extensively raised pastoral cattle herds.

Study area

A cross sectional study was conducted from the late dry season to the early rains of 2017 in two selected States; Oyo and Ogun States of Southwest Nigeria (Figure 1). The study was conducted on cattle herds managed extensively by transhumance and agro-pastoralists.

Target animals

Apparently healthy cattle were screened in this study from randomly selected herds that were traditionally managed by extensive grazing. The animals were fed on communal pasture and shared common water sources. The herds mixed with one another in the day time during grazing and were tethered closely, although separately at noon when they retire for day.

Method

Samples were collected directly from the rectum into sterile universal bottles. The age of sampled cattle was categorized as calf (<1year), young (1-3years) and adult (>3years). The body conditions of the screened cattle were scored as lean, moderate and fat (Nicholson and Butterwort 1986). The breed and sex of each animal together with the name of the herd owner were also documented. The *Faecal* samples were labeled accordingly and were kept in a cool box with ice packs after collection and during transport to the laboratory. The *Faecal* samples were processed at the Department of Microbiology and Parasitology Laboratory, Federal University of Agriculture, Abeokuta, Nigeria. The salt floatation protocol (Ted *et al*. 2007) was utilized in processing the *Faecal* samples and the eggs and oocysts of gastro-intestinal parasites were identified by their morphological features as described by Soulsby, (1982).

Statistical Analysis

Raw data was entered and coded into a Microsoft Excel Sheet (2007); descriptive

Figure 1: Map of Nigeria depicting study areas

statistics was used to summarize data obtained into percentages and IBM SPSS version 20.0 software, was used to analyze the association between gastro-intestinal prevalence and other related parameters in the cattle population studied. The confidence interval was set at 95% and a P-value ≤0.05 was considered to be statistically significant.

Results

342 cattle from 15 extensively managed herds (Table I) were sampled for this study. Of the total cattle sampled, 77 (22.51%) were male and 265 (77.49%) were female. A total of 309 (90.35%) White Fulani cattle and 33 (9.65%) cross breeds were sampled. 31 (9.06%) of the total number of cattle examined were calves, 74 (21.64%) were young and 237 (69.30%) were adults. Based on the body conditions of the 342 cattle sampled, 133 (38.89%) were lean, 173 (50.58%) were moderate and 36 (10.43%) were fat. Using the herds sampled: 36 (10.53%); 41 (12.00%); 24 (7.02%); 22 (6.43%); 16, (4.68%); 24 (7.02%); 13 (3.80%); 29 (8.48%); 31 (9.06%); 14 (4.09%); 7 (2.05%); 12 (3.51%); 32 (9.36%); 23 (6.73%); 18 (5.26%) cattle were sampled from herds A, B, C, D, E, F, G, H, I, J, K, L, M, N and O respectively.

An overall prevalence of 66.1% (226/342) for gastro-intestinal parasites was recorded among the cattle screened in this study. Table II shows the prevalence of gastro intestinal parasites across breed, age, sex and body condition scores of the cattle screened. The prevalence was 72.7% and 65.4% across the breeds for the Cross bred and White Fulani breeds respectively; while prevalences of 61.3%, 66.2% and 66.7% were recorded across the age groups of <1year, 1-3yrs and >3yrs respectively. A prevalence of 63.6% and 66.8% was recorded for male and female respectively across the sexes studied and a prevalence of 61.7%. 69.4% and 66.7% was recorded across body condition scores of lean, moderate and fat BCS respectively.

There was no statistical significance between prevalence and age, sex and breeds. However, a p-value of 0.05 was obtained from the analysis of association between the overall prevalence of GIP and different cattle herds screened (Table III).

Out of the 66.1% of cattle that were positive for GIP, 40.64% had a single parasitic infection while 25.44% of them had multiple parasitic infections (Table IV). Statistical analysis revealed that cattle with a single GIP infection were significantly higher than cattle with mixed GIP infections ($p = 0.000$).

Of the cattle screened 16.67% (57/342) had mixed infections from two parasites, namely, Strongyles and Eimeria, followed by

Table I: Distribution of Gastro Intestinal Parasites by Herds, Sex, Breed and Body Condition Score Herds of cattle

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
No. Observed	36	41	24	22	16	24	13	29	31	14	7	12	32	23	18
Prevalence (%)	10.53	12.00	7.02	6.43	4.68	7.02	3.80	8.48	9.06	4.09	2.05	3.51	9.36	6.73	5.26
Male	12	4	4	2	3	4	8	6	8	5	2	3	3	8	6
Female	24	37	20	20	13	18	5	23	23	9	5	9	29	15	12
W/ F*	26	41	23	21	16	22	11	29	29	14	5	10	32	14	17
Cross	10	0	1	1	1	2	2	0	2	0	2	2	0	9	1
Calves	4	0	7	2	3	2	2	4	2	0	0	0	0	0	3
Young	8	7	34	4	8	2	6	6	19	2	0	0	6	3	5
Adult	24	34	7	16	5	20	5	19	10	12	7	12	26	20	10
Lean	16	7	17	4	11	4	9	12	17	2	0	4	11	9	6
Moderate	19	25	7	16	5	10	3	17	14	12	2	51	5	13	10
Fat	1	9	0	0	0	8	1	0	0	0	5	3	6	1	2

**W/F: White Fulani*

Table II: Association between overall prevalence of gastro-intestinal parasite and studied variables

Variable		No. observed	Positive	X^2	p-value
Breed	Cross	33	24 (72.7)	0.720	0.396
	White Fulani	309	202 (65.4)		
Sex	Male	77	49 (63.6)	0.265	0.607
	Female	265	177 (66.8)		
Age	Calf	31	19 (61.3)	0.354	0.838
	Young	74	49 (66.2)		
	Adult	237	158 (66.7)		
Body Condition	Lean	133	82 (61.7)	2.000	0.368
	Moderate	173	120 (69.4)		
	Fat	36	24 (66.7)		

**Significant, X^2: chi square*

Table III: Association of overall prevalence of GIP infection and different cattle herds screened

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
No. Observed	36	41	24	22	16	24	13	29	31	14	7	12	32	23	18
No. Positive (%)	31 (86.1)	35 (85.4)	16 (66.7)	17 (77.3)	09 (56.2)	14 (58.3)	09 (69.2)	15 (51.7)	16 (51.6)	08 (57.1)	04 (57.1)	07 (58.3)	21 (65.6)	14 (60.9)	10 (55.6)
X^2	23.674														
p-value	0.050*														

**W/F: White Fulani*

Table IV: Association of overall prevalence of GIP infection and of single and mixed infections

	Single	**2-mixed**	**3-mixed**	**4-mixed**	**5-mixed**	**6-mixed**
No. infected (%)	139 (40.64)	57 (16.67)	23 (6.73)	5 (1.46)	1 (0.30)	1 (0.30)
X^2	342.000					
p-value	**0.000***					

**Significant, X^2: chi square*

those with three parasites 6.73%, (23/342) and four parasites 1.46%, (5/342) while only one animal had five and another one had six parasites 0.30% (1/342).

Eleven different *Genera* of parasites were recovered during *Faecal* examinations, namely:Strongyles, Strongyloides, *Nematodirus*, Toxocara (Neoascaris), *Trichuris*, *Fasciola*, *Paramphistomum*, Schistosomum, *Taenia*, *Moniezia* and Eimeria. From this study all the three classes of helminthes (nematodes, cestodes and trematodes) were observed. Nematode eggs were demonstrated in 176/342 (51.46%) of the animals, trematode eggs were found in only 15/342 (4.39%) and cestode eggs in just 9/342 (2.63%). The highest prevalence of parasite distribution was for Strongyle eggs (36.55 %), while *Taenia* spp (0.58 %) and *Trichuris* spp (0.58 %) eggs had the least prevalence. Eimeria oocysts were demonstrated in 137/342 (37.14 %) of the study population (Table V).

Table VI shows the prevalence of gastro intestinal helminthes to be significantly highest among cattle with moderate body condition score (51.4%) compared to lean (39.1%) and fat (33.3%) cattle (p-value = 0.034). This outcome could be substantiated by the large capacity of the body mass of moderate cattle.

Table V: Prevalence of GIP

Gastro-intestinal helminthes	**Number observed (%)**
Nematodes	
Strongyles	125 (36.55)
Strongyloides spp	27 (7.89)
Nematodirus spp	17 (4.97)
Toxocara spp	5 (1.46)
Trichuris spp	2 (0.58)
Total	**176 (51.46)**
Trematodes	
Fasciola spp	7 (2.05)
Paraphistomum spp	5 (1.46)
Schistosomum spp	3 (0.88)
Total	**15 (4.39)**
Cestodes	
Moniezia spp	7 (2.05)
Taenia spp	2 (0.58)
Total	**9 (2.63)**
Protozoa	
Eimeria spp	137 (40.06)
Total	**342**

Table VI: Association between prevalence of GI helminthic infection and body condition score

Variable		No. observed	Positive (%)	X^2	p-value
Body Condition	Lean	133	52 (39.1%)	6.753	0.034*
	Moderate	173	89 (51.4%)		
	Fat	36	12 (33.3%)		

**Significant, X^2: chi square*

Table VII: Association of prevalence of gastro-intestinal helminthic infection and cattle herds

	Herds of cattle														
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**
No. Observed	36	41	24	22	16	24	13	29	31	14	7	12	32	23	18
No. Positive (%)	29 (80.6)	24 (58.5)	11 (45.8)	12 (54.5)	7 (43.8)	9 (37.5)	3 (23.1)	12 (41.4)	8 (25.8)	5 (35.7)	1 (14.3)	7 (58.3)	12 (37.5)	11 (47.8)	2 (11.1)
X^2	43.298														
p-value	0.000*														

**Significant, X^2: chi square*

An analysis of association between prevalence of GI helminthes and different cattle herds (Table VII) revealed a significance of p-value = 0.000 with herd A having the highest prevalence of 80.6% and herd O the least prevalence of 11.1%. This could be attributed to the fact that herd A resides solely within the town while the other herds are transhumant.

Discussion

All the cattle screened are managed under extensive pastoralism in which large percentage of cattle from different herds mix together during the day. This management type increases the degree of pasture contamination leading to a higher infection rate in grazing cattle and the easy spread of diseases due to their mingling together as proven by the overall prevalence of 66.1% that was obtained in this study which corroborates the 62.7% obtained in a previous similar study by Oluwole *et al.* (2016) in Eruwa, Southwestern, Nigeria. All the GIP identified in this study have been previously reported in Nigeria (Anene *et al.*, 1994; Elele *et al.*, 2013; Nnabuife *et al.*, 2013; Adedipe *et al.*, 2014; Okike-Osisiogu *et al.*, 2016; Oluwole *et al.*, 2016) and outside the country by various authors (Rafiullah *et al.*, 2011; Tshering and Dorji, 2013). Two GI parasites namely; Strongyles and Eimeria spp recorded the most prevalence (36.55%, 40.06%) which agrees with their predominant status of 47%, 3.9% and 24.8%, 20.9% in studies carried out in Ethiopia and Cameroon (Regassa *et al.*, 2006; Ntonifor *et al.*, 2013). Also, the higher prevalence of Stronygle eggs observed could be due to the sampling during the late dry season and the early rains of the year.

The predominance of Strongyle parasites in our study also corroborates previous studies in Kenya (Kagari *et al.*, 2010), in Bhutan (Tshering and Dorji, 2013) and in Nigeria (Anene *et al.*, 1994). This could be as a result of the favourable climatic conditions and poor health management of the herds of cattle screened. Also, the Strongyles group of parasites are referred to as the most important of all in tropical and sub-tropical areas (Bricarello *et al.*, 2007; Neves *et al.*, 2014).

Our study further revealed a gastro intestinal helminthes prevalence of 51.46% which is consistent with a report of Pam *et al.* (2013) in the North Central part of Nigeria where a prevalence of 53.77% was recorded. This was higher than 41.6% and 44.39% recorded in Southwestern Nigeria (Adedipe *et al.*, 2014; Takeet *et al.*, 2016) but lower than, 77.1% and 87.41% recorded in Southeastern and Northeastern Nigeria respectively (Okike-

Osisiogu *et al*., 2016; Afolabi *et al*., 2017). The moderate GI helminthes prevalence recorded in this study could be as a result of the rearing system where the cattle examined are extensively raised and a large number of the cattle are tethered close to each other at night for security reasons which aids infection spread. The large number of cattle tethered around an area and the proximity of herds increases the rate of pasture contamination, hence aiding transmission of GI helminthes.

Among the GI helminthes infected cattle in our study, the class nematode had the highest prevalence, followed by trematode and cestode which is consistent with the study carried out by Tshering and Dorji (2013). In tropical and sub-tropical regions where sustenance rations of nutrition exacerbate the damaging effects of infection, morbidity and mortality due to nematode infections are not uncommon (Ademola and Eloff, 2010). The trematode and cestode class that were reported in this study are equally of utmost importance, as some of them (*Fasciola*, Schistosomum and *Taenia*) are capable of causing significant debilitating effects in ruminants, economical loss and are of public health importance.

Also, the statistically significant difference among body condition scores of the cattle screened in relation to prevalence of GI helminthes which revealed moderate BCS to be the one with highest infection, followed by lean and fat BCS is an indicator that the moderate cattle were yet to lose condition because most of the parasites identified have a chronic course in animals. Also, the body mass of moderate cattle increases their capacity to accommodate more GIP. This calls for constant monitoring before they start to deteriorate.

The low prevalence of *Fasciola* sp (2.05%) observed in this study contradicts the prevalence of 22.6% recorded by Oluwole *et al*. (2016), who reported *Fasciola* sp as the highest prevalent infection. However, seasonal differences might be responsible for this variation as the present study was carried out in the late dry season/ early rainy season, when there wasn't sufficient moisture for the propagation of intermediate hosts.

Furthermore, the identification of zoonotic helminthes withpublic health implications such as *Schistosoma*, *Fasciola*, *Taenia* and *Trichuris* is a cause for concern to the *genera*l populace. It is therefore pertinent that the *Faecal* examination of cattle be carried out routinely together with a holistic approach that would take into consideration the parasites, animals, humans and the environment to effectively combat GIP, thereby promoting animal health and food safety.

The high prevalence of single infections followed by a varying number of mixed parasitic infections conforms to a previous study by Nath *et al*. (2016) and the relatively high prevalence (25.44%) of multiple parasitic infections obtained in our study is because of the extensive grazing of the cattle screened. This promotes picking of varying parasites' oocysts on contaminated pasture. The mixed parasitic infections observed in our study may lead to immune-suppression of the host by one of the co-infecting parasites, which may result in increased severity of infection and possibility of further infection by other opportunistic parasites (Su *et al*., 2005; Bandilla *et al*., 2006).

Also, the statistical significance observed between the prevalence of GI helminthes and the different herds examined recorded that herd A had the highest prevalence of GI helminthes in comparison to other herds. These could have beeen because herd A was mainly stationed and grazed within the town unlike the other herds that were transhumant. The transhumant herds over the years most likely might have built resistance to some GI helminthes unlike herd A that doesn't leave the confines of the town in which they are being reared but shares the same communal water and contaminated pasture with other herds that travel through and out of the town seasonally.

Conclusion

This study mirrors what is currently happening in Southwest, Nigeria. To prevent these gastro intestinal parasites from entering the food chain; since they are endemic, some

are zoonotic and widespread across the nation. There is a need to tackle this menace at the level of rearing before the cattle are slaughtered. This will require a concerted effort by all animal health workers and policy makers to ensure that farmers and pastoralists understand the gravity of the negative impact of GIP both on animal and human health, with the possible need for prophylaxis and treatment when required. In addition, the routine monitoring of GIP infection should be enforced and maximally utilized.

Acknowledgement

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Conflict of Interests

Authors declare no conflict of interests

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EVALUATION OF ANTIBIOTIC RESIDUES IN IMPORTED FROZEN CHICKEN THIGH MUSCLES MARKETED IN SOUTHERN BENIN

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Abstract

The international trade in poultry meat and the poultry industry has grown considerably in recent years. The global development of the poultry industry has been followed by a wider use of veterinary drugs, especially antibiotics. However, the presence of antibiotic residues in exported poultry meat is poorly documented. In this study in Benin, residues of amphenicols, beta-lactams, macrolides, streptomycin, sulphonamides and tetracyclines were investigated in chicken thigh muscles imported from Germany, Brazil, France, Italy and the United Kingdom. The results revealed amphenicol residues above the permitted MRLs in 12.5% and 25% of the samples from France and Italy respectively, beta-lactam residues in 37.5% of the samples imported from Brazil, macrolide residues in 37.5% of the samples from the United Kingdom and sulphonamide residues in 100% of the samples from Italy. tetracycline and streptomycin residues were not found in any samples of chicken thigh muscles imported from Germany which were free of all residues. The presence of antibiotic residues beyond the permitted MRLs in exported chicken meat is a global public health concern. Measures should be taken in each importing country and at the international level to enforce the regulations.

Key words: Residues - antibiotic - chicken thigh - imported - Benin

Résumé

Le commerce international de viande de volaille et l'industrie de la volaille se sont beaucoup développés ces dernières années. Ce développement de l'aviculture à l'échelle mondiale s'est aussi accompagné d'une plus large utilisation de médicaments vétérinaires, et surtout d'antibiotiques. Mais la présence de résidus d'antibiotique dans la viande de volaille exportée est mal connue. Dans cette étude faite au Bénin, des résidus d'amphénicols, de bêta-lactamines, de macrolides, de sulfamides de streptomycines et de tétracyclines ont été recherchés dans les muscles de cuisse de poulets importés d'Allemagne, du Brésil, de la France, de l'Italie et du Royaume-Uni. Les résultats ont révélé des résidus d'Amphénicol au-dessus des LMR autorisées dans respectivement 12,5% et 25% des échantillons provenant de France et d'Italie, des résidus de bêta-lactamines dans 37,5% des échantillons importés du Brésil, ceux de macrolides dans 37,5 % des échantillons du Royaume-Uni et ceux de sulfamides dans 100% des échantillons d'Italie. Les résidus de tétracyclines et de streptomycines n'ont été trouvés dans aucun échantillon et les échantillons importés d'Allemagne étaient exempts de tout résidu. La présence de résidus d'antibiotiques au-delà des LMR autorisés dans la viande de poulet exportée constitue un problème de santé publique à l'échelle mondiale. Des mesures devraient être prises dans chaque pays importateur et aussi sur le plan international pour faire respecter les règlementations en vigueur.

Mots clés : Résidus - antibiotique – cuisses de poulets importés - Bénin

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Introduction

The international trade in poultry meat has significantly increased in the last 20 years. In 2010, it was estimated at 11.6 million tonnes, with a growth of 4.8% between 2000 and 2010. Poultry meat exports reached 13 million tonnes in 2017, corresponding to a 2.8% growth compared to 2016. This increase in international poultry meat trade is due to several factors. The demand for poultry meat is increasing because of global economic growth. Moreover, differences in poultry meat production costs between countries and differences in the price of chicken cuts according to importing countries' consumer habits favour trade. Additionally, international tariff concessions and the formation of large groups make poultry meat more accessible to consumers. Ninety percent of the world's poultry meat exports come from Argentina, Brazil, Canada, Chile, the United States, Thailand and the European Union. Chicken meat represents 93% of these exports (France AgriMer, 2012, FAO, 2018).

In this context, developing countries such as Benin, depend on the importation of poultry meat. In Benin, from 2004 to 2014 the annual imports of chicken meat and offals increased from 30,758,552 kg to 134,744,836 kg (INSAE / Benin, 2018). But unlike the European Union or the United States of America (CE, 2010, Donoghue, 2003), most developing countries do not have an efficient quality control system for local or imported foodstuffs of animal origin. In West Africa, for example, only pathogenic microbial agents, pesticide residues and aflatoxins have been included in Regulation 07/2007 / CM / UEMOA on the safety of plants, animals and foodstuffs in the West African Economic and Monetary Union (UEMOA). Residues of veterinary drugs in foodstuffs have not been considered (UEMOA, 2007). Nevertheless, among the health risks faced by consumers in these countries, the risk posed by the presence of veterinary drug residues must be taken into account (Mensah *et al*., 2014b). Veterinary drugs, including antibiotics, are widely used in poultry farming to fight diseases, increase livestock productivity and reduce stress after vaccination or zootechnical actions. The use of veterinary drugs without veterinary controls and especially the non-compliance with drug withdrawal periods leads to the presence of their residues in the foodstuffs of animal origin (Donoghue, 2003, Mensah *et al*., 2014 b, Abdelmoaty, 2015). The potential risks for the consumer related to the presence of antibiotic residues in foodstuffs are many: carcinogenic risks with nitrofurans, allergic risks with beta-lactams and streptomycins, the risk of irreversible spinal aplasia with chloramphenicol, the risk of alteration of intestinal flora with tetracyclines and the risk of selection of antibiotic-resistant bacteria (Lee *et al*., 2001, Chataigner and Stevens, 2003, Sanders *et al*, 2011).

Previous studies in Benin on antibiotic residues in foodstuffs of animal origin involved locally produced reformed layer hen muscles and cows' milk (Mensah *et al*., 2011, Mensah *et al*., 2014a). Although the presence of antibiotic residues has been detected in imported chicken meat in neighbouring Nigeria (Dipeolu and Dada 2005, Omotoso and Omojola 2014), there are no data on the presence of antibiotic residues in imported animal products in Benin. This study aimed to evaluate antibiotic residues in imported frozen chicken thigh muscles marketed in southern Benin.

Materials and Methods

Study area

The study was carried out in two of the most populated municipalities of South Benin, the municipality of Cotonou with 679,012 people and the municipality of Abomey-Calavi with 656,350 people (INSAE / Benin, 2016) where import wholesalers are located (Figure 1). The average annual rainfall of these municipalities varies from 1462 to 2005 mm and their average annual temperature from 24.9 to 31.1°C according to data collected between 2008 and 2010 (INSAE / Benin, 2012).

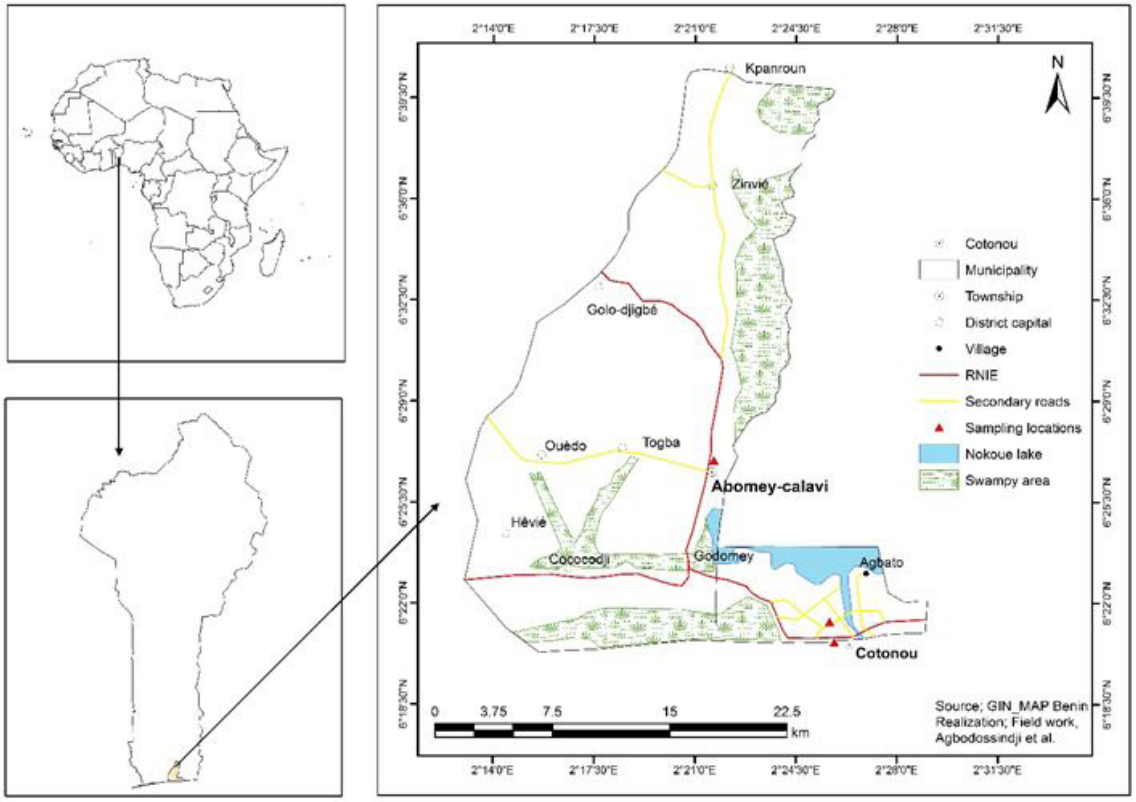

Figure 1: Study area

Sampling

The samples of frozen chicken thighs were collected in July 2016. One (01) carton of frozen chicken thighs by the available countries of origin (Brazil, France, Germany, Italy and the United Kingdom) was randomly chosen from import wholesalers operating in the municipalities of Cotonou and Abomey-Calavi in southern Benin. Each carton contained twenty-four (24) chicken thighs that were grouped into eight (08) batches of three (03) thighs. Forty (40) batches of three chicken thighs in total were thus constituted for the detection of antibiotics residues.

Detection of antibiotic residues

The chicken thighs of each batch were de-boned, de-fatted and mixed and then 100g of lean muscle tissue was collected for the detection of amphenicols, beta-lactams, macrolides, streptomycins sulphonamides and tetracyclines residues in the Laboratoire Central de Contrôle de Sécurité Sanitaire des Aliments (LCSSA) of Benin by the Charm II 7600 method (Charm Sciences Inc., 2014). The Charm II test positivity limits and the Maximum Residue Limits (MRLs) recommended by the Codex Alimentarius and the European Union for each antibiotic family in muscle are shown in Table 1.

Statistical analyses

The percentages of positivity or negativity to antibiotic residues in the chicken thigh muscles were calculated according to the countries of origin and the antibiotics families using the following formula:

$$P_i = \frac{n_i}{N} \times 100$$

With P_i as the percentage of positivity or negativity to the antibiotic residues for the country of origin (i), or the antibiotics families (i); ni as the number of chicken thigh batches

declared positive or negative to antibiotic residues for the country of origin (i) or the antibiotics families (i) and N as the total number of chicken thigh batches tested.

Fisher's exact test was used to compare the variations in positivity or negativity by country of origin or by family of antibiotics. All analyses and graphics were performed in the R 3.5.0 software environment (R Core Team, 2018).

Table 1: Charm II test positivity limits and MRL (µg / kg) for the different antibiotic families in muscle

Antibiotic families	Charm II test positivity limits (µg/kg)	MRLs Codex Alimentarius (µg/kg)	MRLs European Union (µg/kg)
Amphenicols	0.3	None	None
Beta-lactams	50	50	50
Macrolides	50 to 400	100 to 200	75 to 200
Sulphonamides	100	100	100
Streptomycins	500	600	500
Tetracyclines	100	200	100

Source: Charm Sciences Inc. (2014); FAO/WHO, 2018 ; CE, 2010.

Results

The proportion of chicken thigh muscles positive to antibiotic residues varied significantly by the country of origin and antibiotic family (P <0.05) as shown in Figures 1 and 2. Thus, regardless of the country of origin, sulphonamides residues were the most prevalent in chicken thigh muscles imported in Benin with 20% positive versus 7.5% for amphenicols, beta-lactams and macrolides, respectively. Regardless of the family of antibiotics, chicken thigh muscles from Italy contained the most residues with 100% positive versus 35.5% for Brazil and the United Kingdom, 12.5% for France and 0% for Germany, respectively.

On the other hand, when countries of origin were grouped by region, the proportion of chicken thigh muscles positive for antibiotic residues (37.5%) was statistically similar (P = 0.869> 0.05) between South America and the European Union as shown in Figure 3.

The comparison of the percentages of imported chicken thigh muscles positive for the different antibiotic families' residues by the country of origin (Table 2) shows that no residues were found in the chicken thigh muscles imported from Germany. Similarly, no *streptomycin* or tetracycline residues were found in the chicken thigh muscles regardless of the country of origin.

On the contrary, residues of *Amphenicols* were present in 12.5% and 25% of the chicken thigh muscles imported from France and Italy respectively; beta-lactam residues were detected in more than 33.3% of the chicken thighs from Brazil; macrolide residues were found in more than 33.3% of the chicken thigh muscles from the United Kingdom and sulphonamides residues in all the chicken thigh muscles.

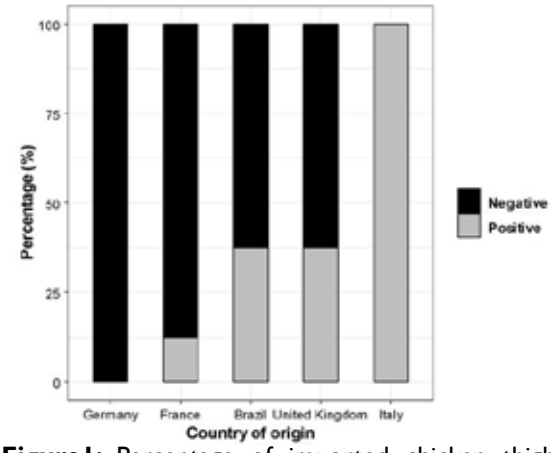

Figure1: Percentage of imported chicken thigh muscles positive for antibiotic residues by country of origin in Southern Benin

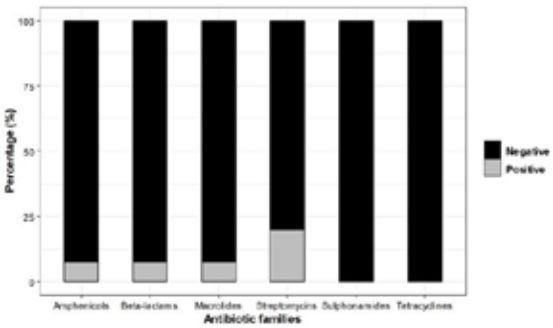

Figure 2: Percentage of imported chicken thigh muscles positive for antibiotic residues by antibiotic families in Southern Benin

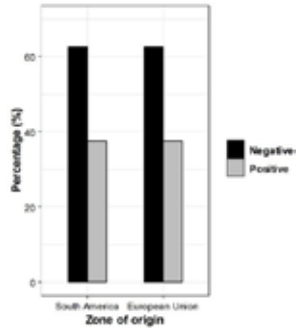

Figure 3: Percentage of imported chicken thigh muscles positive for antibiotic residues by zone of origin in Southern Benin

Table 2: Percentage of imported chicken thigh muscles positive for different antibiotic families' residues by the country of origin in Southern Benin

Antibiotics families	**Country of origin**				
	Germany	**Brazil**	**France**	**Italy**	**United Kingdom**
Amphenicols	0.0	0.0	12.5	25.0	0.0
Beta-lactams	0.0	37.5	0.0	0.0	0.0
Macrolides	0.0	0.0	0.0	0.0	37.5
Streptomycins	0.0	0.0	0.0	0.0	0.0
Sulphonamides	0.0	0.0	0.0	100.0	0.0
Tetracyclines	0.0	0.0	0.0	0.0	0.0

Discussion

Except for Brazil all the countries from where chicken thighs used in this study were imported are members of the European Union (EU). These EU countries and Brazil are supposed to comply with EU or Codex Alimentatrius standards for antibiotics MRLs (EC, 2010, FAO / WHO, 2018). But antibiotic residues were detected beyond the recommended MRLs in 12.5% to 100% of the chicken thigh muscles from all these countries except Germany.

Amphenicols, for example, for which no MRL is established and which are banned in the EU, were detected in 12.5% and 25% of the chicken thigh muscles from France and Italy, respectively. This suggests that amphenicols are still clandestinely used in these countries. Residues of other antibiotics such as beta-lactams, macrolides and sulphonamides were found above the recommended MRLs in the chicken thigh muscles from Brazil, United Kingdom and Italy respectively in proportions ranging from 37.5% to 100%. These results suggest that withdrawal periods after the use of these antibiotics were not respected in these countries of origin before the slaughter and the export of the thighs of these chickens. The possibility that chicken muscles containing antibiotic residues beyond the recommended MRLs may be intentionally exported to developing countries such as Benin could also be considered.

The overall proportions of chicken thigh muscles positive for beta-lactam (7.5%) and sulphonamide (7.5%) residues in this study were less than the 10% found by Elbagory *et al*. (2016) for ampicillin and 64% obtained by Karmi (2014) for sulphonamides in frozen chicken muscles imported into Egypt. These differences could be due to differences in the

samples of chicken muscles tested in the two studies. Beta-lactams have also been detected in imported frozen chicken meat marketed in Abeokuta, Nigeria that neighbours Benin (Olajumoke *et al*., 2016).

All chicken thigh muscles, in this study, were free of streptomycin and tetracycline residues. Nevertheless, *Tetracycline* residues have previously been found in chicken meat imported into Nigeria via Benin and marketed in Lagos and Ibadan (Adetunji *et al*., 2012). Likewise, tetracycline residues have been identified in 14% of the reformed layer hen meat imported and marketed in Ogun, Lagos and Oyo States in Nigeria (Dipeolu and Dada, 2005). More recently, still in neighbouring Nigeria, residues of *Tetracycline*s and *streptomycin*s were detected in frozen chicken meat marketed in Abeokuta, Ogun State (Olajumoke *et al*., 2016). Quinolones were also detected in 52.53% of imported frozen broiler muscle samples sold in Ibadan (Omotoso and Omojola, 2014). Elsewhere in Africa, particularly in Egypt, antibiotic residues studies revealed the presence of tetracyclines, quinolones and aminoglycosides residues in 48%, 16% and 12%, respectively of imported frozen chicken muscle samples (Karmi, 2014) and oxytetracycline residues in 35% of the analysed samples (Elbagory *et al*., 2016). The absence of tetracyclines and streptomycin in chicken thigh muscles imported and marketed in Benin in this study could be attributed to a sampling bias. Otherwise, the quinolones and aminoglycosides not investigated in this study could be present in the imported chicken leg muscles in Benin and future studies should take this into account.

Antibiotic residues detected in the imported frozen chicken muscles in this study and the studies cited above are the most frequently found in animal foods in Africa (Darwish *et al*., 2013). These are tetracyclines (41.17%), beta-lactams (17.69%), quinolones (5.88%), macrolides (5.88%) and chloramphenicol (5.88%). These antibiotics, with sulphonamides, are the most commonly used in poultry farming (Wouembe 2013, Dosso 2014).

The presence of antibiotic residues in imported chicken muscles in Africa poses a serious public health problem. Major poultry exporting countries should monitor compliance with antibiotic residue standards for both locally consumed poultry (EC, 2010, Donoghue, 2003) and exported poultry. In addition, African countries need to have effective systems for controlling antibiotic residues in imported foods (Mensah *et al*., 2014b, Abdelmoaty, 2015) and lobbying for compliance with Codex Alimentarius standards in the international poultry trade.

Conclusion

In this study, 20% of all the chicken thigh muscle samples imported from different countries (Brazil, France, Italy and the United Kingdom) were sulphonamides positive and 7.5% were positive for beta-lactams, macrolides and amphenicols. The levels of antibiotics detected were beyond the Codex Alimentarius MRLs. tetracycline and streptomycin residues were absent from all chicken thigh muscle samples. No antibiotic residues were present in chicken thigh muscle samples imported from Germany. These results show that international standards for antibiotic use and MRLs are not being met in some of these chicken thigh exporting countries. Poultry importing countries such as Benin therefore need to have a system for controlling antibiotic residues in imported poultry meat to ensure the protection of their consumers.

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INDIGESTIBLE FOREIGN BODIES IN SLAUGHTERED CATTLE (OCCURRENCE AND SEASONALITY) IN AN ABATTOIR IN SOUTH-EASTERN NIGERIA.

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Abstract

Four hundred and fifty animals destined for slaughter were selected using simple random sampling and examined for foreign bodies. Of the animals sampled, 316 (70.2%) had indigestible foreign bodies in their rumen and/ or reticulum. Female animals had more foreign bodies (85.7%) than males (69.5%). More animals had foreign bodies in their rumen (67.6%) than in their reticulum (1.3%). It was discovered that the older the animal is, the more foreign bodies it accumulated as 65.6%, 67.1% and 86.8% of animals less than 3 years, 3- 5 years and greater than 5 years had foreign bodies respectively). In this study, 43.9% of the animals with very good body conditions had foreign bodies compared to 100% of those with very poor body conditions. Foreign bodies were recovered from more cattle in the dry season (78.7%) than in the rainy season (61.8%). A variety of foreign bodies were encountered in this study and they included feed sack threads, polythene, raffia, cloth, sachets, rope, metals, stones, fruit seeds, battery heads and plastics. The age of the animals, location of the foreign bodies, body conditions of animas and seasonal variations were found to be statistically significant at 0.006, 0.000, 0.000 and 0.000 respectively.

Keywords: Abattoir environment, foreign bodies, rumen, reticulum, cattle

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Introduction

Nigeria has a population of about 13.9 million cattle (Lawal and Adebowale, 2012). Cattle are a source of high-quality protein (meat and milk) and also contribute to the economic welfare of people by providing hides, skins, manure, draught power and traction for agricultural purposes thus increasing the productivity of smallholdings (Torr *et al*., 2003). They are also a 'living savings bank', serving as a financial reserve for periods of economic distress and crop failure and as a primary source of cash income (ILRI, 1999).

Cattle are more susceptible to the foreign body syndrome than small ruminants because they do not use their lips for prehension and are more likely to eat chopped feed. The lack of oral discrimination in cattle may lead to ingestion of foreign bodies which would be rejected by other species (Desiye and Mersha, 2012). The ingestion of foreign bodies is mainly related with nutritional deficiencies and feeding management and causes various problem in different organs of the animal, mainly in the rumen and reticulum (Jones *et al*., 1997). Diseases of the rumen and reticulum are of great economic importance because of severe losses in productivity of the animals sometimes leading to the death of the animals (Radostits *et al*., 2007).

The problems that are caused vary with how long the foreign body has been present, the location of the foreign body, and the degree of obstruction caused as well as problems associated with the type of foreign body. The presence of foreign bodies in the rumen and reticulum also hampers the absorption of volatile fatty acids (VFA) and consequently leads to a reduction in the rate of animal fattening (Igbokwe *et al*., 2003). The perforation of the wall of the reticulum allows leakage of ingesta and bacteria which contaminates the peritoneal cavity, resulting in local or diffuse peritonitis. The swallowed objects can also penetrate the pleural cavity causing pleuritis and pneumonitis and the pericardial sac causing pericarditis (Cavedo *et al*., 2004).

Cattle in Nigeria perform below the expected potential due to poor management, prevalent diseases and poor genetic performance (Abebe, 1995). Investigation of the problems that affect these cattle in the study area would help to provide the solutions needed to improve their potential and increase productivity.

The significance of this study is to draw attention to the role of environmental pollution on the prevalence of foreign bodies and its health risks and how it contributes to reduced production and sometimes leads to the death of affected cattle.

Materials and Methods

Study area and design

A cross sectional study was conducted from January, 2016 to June, 2016 to determine the prevalence of foreign bodies in the rumen and reticulum of slaughtered cattle and to identify the types of foreign bodies and their associated risk factors. The study was conducted at Ubakala Abattoir, Umuahia South Local Government Area (LGA) of Abia State, Nigeria. The slaughterhouse used for this study is owned by the Abia State Government and managed by the State Ministry of Agriculture and Natural Resources.

A total of 450 cattle were used for this study a total of 225 cattle were sampled during the dry season and 225 were sampled during the wet season.

The selected cattle selected were marked and tracked through the slaughter process.. The animals were examined to determine the sex, age, breed, the body condition and source before slaughter.

Age was determined by the dentition of the animal based on the appearance (eruption) and wear of the incisor teeth as previously described (Otesile and Obasaju, 1982).

The body condition score of each selected animal was evaluated by observation and feeling the level of muscle and fat deposition over and around the vertebrae in the loin region as described by Thompson and Meyer (1994).

After slaughter, flaying and evisceration, the rumen and reticulum were incised and visually examined for the presence of foreign bodies. Foreign bodies recovered from each rumen and reticulum were washed, identified, dried, weighed and recorded with respect to type and location.

Data obtained was analyzed using IBM SPSS version 20 for percentages and frequencies. One way ANOVA was used to determine the statistical significance of the different variables at 0.05 level.

Results

Four hundred and fifty (450) cattle slaughtered at the Ubakala abattoir were sampled for the presence of foreign bodies in the rumen and reticulum. The sampling was done in during the wet season and in the dry season Two hundred and twenty five (225) cattle were sampled in each of the seasons.

Out of the 450 cattle sampled, 298 (69.5%) males and 18 (85.7%) females had foreign bodies.

The foreign bodies recovered ranged from raffia, ropes, seeds, polythene, metals, cloth, stones, sachets, battery heads and threads from feed sacks (Figures. 1, 2, 3, 4, 5, 6 and 7).

Figure 1: Stones recovered from the rumen of cattle

Figure 2: Metal objects recovered from the reticulum

Figure 3: Raffia recovered from the rumen of cattle

Figure 4: Compact polythene bags recovered from the rumen of cattle

Figure 5: Loose polythene bags recovered from the rumen of cattle

Figure 6: Pieces of cloth recovered from the rumen of cattle

Figure 7: Ropes recovered from the rumen of cattle

Figure 8: Feed sack threads (FST)

Figure 9: Stones covered with feed sacks used for tethering animals

Figure 10: Feed sacks containing yams from a vehicle used for transporting cattle

Table 1: Prevalence of rumen and reticulum foreign bodies in cattle slaughtered at Ubakala abattoir in association with Sex

Sex	Number sampled	Positive cases	Prevalence (%)
Female	21	18	85.7
Male	429	298	69.5
Total	**450**	**316**	**-**

Table 2: Prevalence of foreign bodies in cattle slaughtered at Ubakala abattoir in association with type of Foreign Bodies present

Type of foreign body	Number recovered	Prevalence (%)
Feed sack thread	250	54.8
Polythene bags	68	14.9
Raffia	39	8.6
Cloth	31	6.8
Sachets	22	4.8
Rope	15	3.3
Metals	11	2.4
Stone	9	2
Fruit seeds	9	2
Battery head	1	0.2
Plastic	1	0.2
Total	**456**	

The most prevalent (54.8%) foreign body in the sampled animals was thread from feed sacks (FST). The other types of foreign bodies found were polythene bags (14.9%), raffia (8.6%), cloths (6.8%), sachets (4.8%), ropes (3.3%), metals (2.4%), stones (2.0%), fruit seeds (2.0%), battery heads (0.2%) and plastics (0.2%).

The prevalence of foreign bodies in association with age is indicted in table 3. The lowest occurrence was found in the younger animals (1-3 years) while the highest occurrence was found in animals greater than 5 years of age.

The prevalence of foreign bodies found in the rumen was greater than in the reticulum.

Table 3: Prevalence of rumen and reticulum foreign bodies in cattle slaughtered at Ubakala abattoir in association with Age

Age	Number sampled	Positive animals	Prevalence (%)
< 3	49	32	65.3
3-5	325	218	67.1
>5	76	66	86.8
Total	**450**	**316**	**-**

Table 4: Prevalence of rumen and reticulum foreign bodies in cattle slaughtered at Ubakala abattoir in association with body conditions of the animal.

Body conditions	Number sampled	Positive cases	Prevalence
Very good	41	18	43.9
Good	243	152	62.6
Satisfactory	156	136	87.2
Poor	9	9	100
Very poor	1	1	100
Total	**450**	**316**	

Table 5: Prevalence of rumen and reticulum foreign bodies in cattle slaughtered at Ubakala abattoir in association with lodgment site

Location	Number of foreign body	Prevalence (%)
Absent	134	29.6
Rumen	304	67.6
Reticulum	6	1.3
Both	6	1.3
Total	**450**	**100**

Table 6: Prevalence of rumen and reticulum foreign bodies in cattle slaughtered at Ubakala abattoir in association with Season

Season	Number sampled	Positive cases	Prevalence (%)
Dry season	225	177	78.7
Rainy season	225	139	61.8
Total	**450**	**316**	**-**

P = 0.000

Table 6a: Seasonal variation of foreign bodies with respect to location

Season	Number sampled	Location of foreign body	Frequency	Prevalence (%)
Dry season	225	Rumen	166	73.8
		Reticulum	5	2.2
		Both	5	2.2
Rainy season	225	Rumen	138	61.3
		Reticulum	1	0.4
		Both	1	0.4
Total	**450**		**316**	**-**

Table 6b: Seasonal variation of foreign bodies in cattle slaughtered at Ubakala abattoir with respect to body condition

Season	Number sampled	Body condition score	Frequency	Prevalence (%)
Dry season	225	Very good	10	5.6
		Good	96	54.2
		Satisfactory	67	37.9
		Poor	3	1.7
		Very poor	1	0.6
Rainy season	225	Very good	8	5.8
		Good	85	61.2
		Satisfactory	45	32.3
		Poor	1	0.7
		Very poor	0	0
Total	450		316	

Table 6c: Seasonal variation of rumen and reticulum foreign bodies in cattle slaughtered at Ubakala abattoir in association with Age

Season	Number sampled	Location of foreign body	Frequency	Prevalence (%)
Dry season	225	< 3	20	8.9
		3-5	113	50.2
		> 5	43	19.1
Rainy season	225	< 3	12	5.3
		3-5	105	46.7
		> 5	23	10.2
Total	**450**		**316**	

Discussion

The overall prevalence of foreign bodies in the sampled cattle was 70.2% (316/450). Because cattle have a poor selective grazing adaptation (Westwood 2011), a prevalence rate of 70.2% in this study is not extraordinary. The high prevalence observed in this study can be attributed to differences in animal management systems and the extent of waste management both in the urban and semi urban areas and in the grazing areas.

The high prevalence of foreign bodies found in the females may be attributed to hormonal changes and increased appetite due to nutritional demands during estrus, pregnancy and lactation in them (Remi-Adewummi *et al*, 2004). It could also be due to the fact that the female animals are kept longer than the males during breeding due to their longer lifespan (Mushonga *et al*, 2015). The difference in the prevalence between the females and males was not statistically significant (p = 0.112).

The high prevalence of FST has not been reported previously. This may be due to its use in tethering in the lairages where large stones are covered with feed sacks (Figures 8 and 9). In addition, the animals may have been exposed to FST while in transit because it was noted that some of them were transported together with yams bagged in feed sacks (fig 10).

In this study, it was noted that the older the animal, the higher the prevalence of foreign bodies found in them. This finding agrees with Berrie *et al*, 2015, Desiye and Mersha 2012 and Rahel 2011. The presence of foreign bodies in older animals may be due to a gradual accumulation over the years. The difference in the occurrence of foreign bodies between the age groups was statistically significant (p = 0.006).

The occurrence of foreign bodies was directly proportional to the body conditions of the animals.

This could be attributed to either the animal losing weight after being exposed to foreign bodies or the interference of foreign bodies with the absorption of volatile fatty acids thus causing reduced weight gain (Tesfaye and Chanie 2012, Rahel 2011, Ismael 2007, Remi- Adewummi., 2004). The difference in the occurrence of foreign bodies in animals with different body conditions was statistically significant (p = 0.000).

This result agrees with Desiye and Mersha (2012) who reported that from 64 positive cases of foreign bodies, 49 (79.7%) were detected in the rumen. This also agrees with Remi-Adewunmi *et al*. (2004) who found 58.5% in the rumen and 19.3 % in the reticulum of Achai Cattle. This discovery has also been reported in small ruminants by Igbokwe *et al*. (2003); Remi – Adewummi *et al*. (2004) and Tiruneh and Yesuwork (2010).

The high prevalence of foreign bodies in the rumen may be due to the fact that ingested food first gets to the rumen and most indigestible materials do not progress to other stomach chambers (Berrie *et al*., 2015; Borden *et al*., 2015). This finding could also be attributed to different factors such as the

larger rumen volume, cumulative size and the material composition of the foreign bodies. Materials such as metals and sharp objects tend to localize preferentially in the reticulum (Radostits *et al*., 2007). There was a significant association between the presence of foreign bodies and their location (p= 0.000).

The majority (78.7%) of the foreign bodies occurred during the dry season. Seasonal variation was found to be a crucial factor in the prevalence of foreign bodies in slaughtered cattle. This factor has been reported by (Rahel 2011; Abebe and Nuru 2011; Mohammed 1989; Igbokwe *et al*., 2003; Sanon *et al*, 2007; Robbins *et al*., 1995) in different locations.

Foreign bodies were more prevalent during the dry season than in the rainy season. This may be due to the greater abundance of forage during the wet season than in the dry season.

Conclusion

The prevalence of foreign bodies observed in the rumen and reticulum of cattle slaughtered in Ubakala Abattoir was high at 70.2%. It is common in most developing countries due to the poor standard of animal management.

The statistical associations between the presence of foreign bodies and age, location of foreign bodies, body condition score and season were statistically significant.

The types of foreign bodies detected in this study were feed sack thread, polythene, raffia, cloth, sachet, rope, metals, stone, seeds, battery head and plastic. This problem is of a great economic importance in Nigeria.

Thus based on the above conclusions, the following recommendations are suggested.

1. Cattle transportation guidelines should be adhered to strictly.
2. The lairages should be properly constructed and made conducive for animals.
3. Animal owners and farmers should be educated on the economic importance of foreign body ingestion in order to maintain production.
4. The abattoir and animal grazing environments should be kept clean and hygienic at all times and environmental pollution avoided.

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A CROSS SECTIONAL STUDY ON THE PREVALENCE OF ECTOPARASITES IN GALLUS *GALLUS DOMESTICUS* (DOMESTIC CHICKEN) IN DUTSINMA LOCAL GOVERNMENT AREA KATSINA STATE

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Abstract

A cross sectional study was conducted from January 2017 to July 2017 to estimate the prevalence of ectoparasites infestations in Dutsinma Local Government Area (LGA) of Katsina State as well as to assess the effects of host related risk factors (sex, coat colour, breed and age). Ectoparasite samples were randomly taken from 944 chickens and were examined by close inspection with naked eyes and magnifying hand lens. Out of the total chickens examined, the following species of ectoparasites were seen: *Echidnophaga gallinacean* (flea), *Menacanthus stramineus* (Lice), *Argaspersicus* (tick) and *Musca domestica* (House fly) with aprevalence of 0.53%, 0.32%, 4.24% and 18.01% respectively. The effect of host related risk factors on the prevalence of ectoparasite infestations did not show any significant differences (P>0.05) between sex, age group, coat colour and systems of production. The observed results of this study suggest that ectoparasite infestation is dominant in the study area. Therefore, effective preventive and control measures need to be instituted to mitigate the menace of ectoparasites in Dutsinma LGA.

Keywords: Chickens, *Dutsinma*, *Ectoparasite*, Infestation, *Katsina*, Prevalence

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Introduction

Local chickens, guinea fowls, geese and turkeys have been used in small scale indigenous poultry production because of the diverse roles it plays. The sale of eggs and live birds at urban and rural markets is perhaps the only source of cash earnings available to rural families (Nwangu, 2002). Despite the presence of large numbers of chickens in Nigeria, their contribution to the national economy or the benefit realized from domestic chickens is very limited due to disease and nutritional limitations (Smith, 1990). Ectoparasites hamper poultry production as they affect the health, growth and productivity through their feeding habits; by sucking their blood, tissue fluid and transmitting deadly pathogens (Yeshitila *et al*., 2011). Ectoparasites play an important role in reducing the total poultry production potential of the country. Parasites are common in tropics, where poor husbandry practices and climatic conditions are favorable for the development of the parasites (Abebe *et al*., 1997). *Ectoparasitism* negatively affects the productivity potential of the local free-range chickens since they either compete for feed or cause distress to the chickens (Sabuni *et al*., 2010). Moyer *et al* (2002) noted that a parasite's potential effect, or "pressure", can influence the life history strategy of its host. In environments with high parasite pressure, hosts invest more in anti-parasite defense, which may limit their investment in other life history components, such as survival (Moyer *et al*., 2002) and production. Most ectoparasites (for example, lice), stay close to the host during their entire life cycle while others move from one host to the next quite frequently such as ticks and mites (Yacob *et al*., 2009). Ectoparasites cause intense pain, irritation, slow weight gain, decreased egg production, and *genera*l poor and ill health (Urquhart *et al*., 1996, Kaufman, 1996). Lice are the most common ectoparasites of poultry, causing major economic loss to the productivity of animals (Fabiyi, 1980; Alamargot *et al*., 1985). Ectoparasites usually consume dead cells of the skin and tissue fluids, suck blood and cause irritation to the birds, which adversely affects their economic productivity (Mullen and Durden, 2002; Permin *et al*., 2002; Mungube *et al*., 2006; and Nyoni and Masika, 2012). Reports have shown that mortality due to parasitic diseases is higher than that attributed to some poultry viral infectious diseases such as Newcastle disease and fowl pox disease (Nnadi and George, 2010; Opara *et al*., 2014). The presence of fleas is *genera*lly associated with skin disorders (dermatitis), pruritus, severe itching and allergic reactions in infested hosts (Koutinas *et al*., 1995). Ectoparasites damage feathers and cause skin lesions, resulting in reduced performance of adult chickens and direct harm to young chicks (Arends, 2003). Despite their devastating effects, ectoparasites have received little attention in almost all the production systems. Hence, this study sought to determine the magnitudes of such parasites and identify their types with a view to proffering appropriate control methods. The study was designed to answer the question, why are most of the extensively reared birds infested with ectoparasites. The objective of the study was to determine the prevalence of ectoparasites in local chickens and to assess the relationship between host related risk factors (age group, sex and coat colour) and ectoparasite prevalence in local chicken in the study area.

Materials and Methods

The study was carried out in Dutsinma Local Government Area, Katsina State, Nigeria. Dutsinma LGA lies on latitude 12°26'18"N and longitude 07°29'29"E. It is bounded by Kurfi and Charanchi LGAs to the north, Kankia LGA to the east, Safana and Dan-Musa LGAs to the west, and Matazu LGA to the southeast.

Dutsinma LGA has a land size of about 552.323 km2 (203sqm) with a population of 169, 829 people as at the 2006 national census (Federal Republic of Nigeria, 2012). The people are predominantly farmers, cattle rearers and traders. The climate of Katsina State is the tropical wet and dry type (tropical continental climate). Rainfall is between May and September

with a peak in August. The average annual rainfall is about 700 mm. The pattern of rainfall in the area is highly variable. The mean annual temperature ranges from 29°C – 31°C. The highest air temperature normally occurs in April/May and the lowest in December through February. Evapo-transpiration is *general*ly high throughout the year. The highest amount of evaporation occurs during the dry season. The vegetation of the area is the Sudan Savanna type which combines the characteristics and species of both the Guinea and Sahel Savanna (Abaje, 2007; Tukur *et al*., 2013).

Sample collection, processing and examination

A cross sectional study was conducted for a period of six months. The study was carried out between January and June 2017 to estimate the prevalence of poultry ectoparasite infestations in Dutsinma. Two livestock markets (weekly market and Hayin-gada poultry market), five villages (Kagara, Ruwangamji, Shanga, Sabongari and Farinkasa), twenty-eight households and five commercial farms (Kofa, Banu, Federal University Livestock Farm, Garhi and Badole farms) were visited for sample collection of ectoparasites. A purposive sampling was used based on the consent of the owners of the chickens. A total of 944 chickens were selected and examined irrespective of the sex, age, groups and species of the chicken for the presence or absence of ectoparasites. Screening for ectoparasites involved a thorough examination of the body of the birds including the head, cloacal, brachial, ventral, and femoral areas as was carried out by Nwangu (2002). Those with ectoparasites were identified and recorded. Similarly, those without ectoparasites were recorded. Samples taken from birds were examined by close inspection with naked eyes and magnifying lens as described by Nwangu (2002). Ectoparasites found on the body of the birds were collected in sample bottles containing 70% alcohol. Collected samples were transported to the Entomology laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State for identification. The ectoparasites were dehydrated in a series of alcohol dilutions of 80%, 90% and 100% before being cleared in xylene and mounted on a slide. Finally, the parasites were identified according to their morphological characteristics using entomological keys using a light microscope (Nwangu, 2002).

Analysis of Results

Ectoparasiteprevalence was calculated with the formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of birds that were identified with a particular ectoparasite}}{\text{Total number of birds sampled during the study period}} X100$$

Raw data and results of parasitological examination were entered into a Microsoft Excel sheet program and exported to SPSS version 20® for analysis. Pearson's Chi square (χ^2) was used to determine the statistical strength of association between categorical variables, host related risk factors and prevalence of ectoparasites infestation. Values with $P<0.05$ were considered significant at 95% Confidence Interval (CI).

Results and Discussion

Out of the total 944 chickens sampled in Dutsinma L.G.A, Katsina State, the following species of ectoparasites were identified: *Echidnophaga gallinacea* (Flea), *Menacanthus stramineus* (lice), *Argas persicus* (tick) and *Musca domestica* (house fly)as shown in table 1.

This study showed that adult chickens with a prevalence of 20.13% were more infested by ectoparasites than growers with a prevalence of 2.97% as shown in table 2.The higher prevalence in adult chickens was associated with the fact that most of the chickens sampled were layer birds kept for egg production. Also, due to the poor management in the layer farms, house flies constituted the most prevalent ectoparasite observed (table 2).

Table 1: Prevalence of Ectoparasites in Domestic Chickens in Dutsinma LGA

Type of ectoparasite	Number of chickens sampled	Number of positive sample	Prevalence (%) (y/944) x 100
Ticks	944	40	4.24
Lice	944	3	0.32
House fly	944	170	18.01
Fleas	944	5	0.53
Total		**218**	**23.10**

Table 2: Ectoparasites Prevalence by Age Group of Chickens in Dutsinma LGA

Age Group	Number of chickens sampled	Number of positive sample	Prevalence (%) (y/944) x 10 0	Chi Square (χ^2) value
Young	12	0	0.00	4.512
Grower	119	28	2.97	
Adult	813	190	20.13	
Total	**944**	**218**	**23.10**	

P = 0.10 at 95% CI

This finding agrees with the works of Biu *et al*., 2007 in Nigeria and that of Permin *et al*. 2002 in Zimbabwe, who reported that Adult chicken were more infested as compared to younger chickens. Adult chickens may have had higher prevalence due their gregariousness relative to growers and chicks thus making them more susceptible. Also, growers and chicks have limited knowledge of their environments and as a result shuttle less distances (Nnadi and George 2010). We cannot advance reasons as to the variation in the prevalence of the various ectoparasites beyond the fact that this may be habitat related.

Brown coloured chicken which were under intensive management suffered only houseflies' disturbance with a prevalence of 19.60%. Mixed coloured chickens (1.80%) haboured more ectoparasites than single coloured chickens (white = 0.64%, black = 1.06%). The high infestation of ectoparasites in mixed coloured chickens may be due to the ability of the parasites to burrow and camouflage more in mixed colours than black and white colours. This camouflage may have enabled the parasites to live and multiply in mix coloured chickens. This agrees with Bala *et al*., 2011 who reported that mixed coloured chickens were more infested than single coloured chickens.

With regards to sex as a risk factor, there was a significant difference in the rate of infestation; female chickens (21.40%) were more infested than male chickens (1.70%). This finding is in tandem with that of Bala *et al*., 2011 who reported that female chickens were more infested than male chickens. However, significant difference was reported in ectoparasite infestations between male and female chickens by Belihu *et al*., 2009 and Tolossa *et al*., 2009 who reported that cocks were more infested than hens. The higher prevalence of ectoparasites in the female chickens may have been as a result of the stationary state of the females during incubation which makes them more susceptible to ectoparasite infestations. In addition, the female chickens may emit some smell which may attract the parasites during the incubation period (Bala *et al*., 2011).

The 170 positive samples from the intensively reared chickens suffered disturbance from house flies alone and had no other form of ectoparasite infestation (Table 5). The invasion of houseflies was as a result of poor sanitation of the poultry houses. In this study, other types of ectoparasite infestation (mites, fleas, lice and ticks) were not associated with the intensively reared chickens but was found with the semi-intensively and the extensively reared chickens.

Table 3: Ectoparasite Prevalence by Coat Colour of the Chickens Sampled

Age Group	Number of chickens sampled	Number of positive sample	Prevalence (%) (y/944) x 10 0	Chi Square (χ^2) value
White	55	6	0.64	14.986
Black	13	10	1.06	
Brown	746	185	19.60	
Mixed	130	17	1.80	
Total	**944**	**218**	**23.10**	

P = 0.10 at 95% CI

Table 4: Ectoparasite Prevalence by Sex of the Chickens Sampled

Age Group	Number of chickens sampled	Number of positive sample	Prevalence (%) (y/944) x 10 0	Chi Square (χ^2) value
Male	56	16	1.70	0.291
Female	888	202	21.40	
Total	**944**	**218**	**23.10**	

P = 0.10 at 95% CI

Table 5: Ectoparasite Prevalence in Relation to Management System

Age Group	Number of chickens sampled	Number of positive sample	Prevalence (%) (y/944) x 10 0	Chi Square (χ^2) value
Intensive	780	170	18.01	3.281
Semi-intensive	78	24	2.54	
Extensive	86	24	2.54	
Total	**944**	**218**	**23.10**	

P = 0.10 at 95% CI

Arend, 1997 noted that management could be a contributing factor to the type of ectoparasites that are predominating in chicken houses.

With regards to the risk factors examined, the prevalence of ectoparasite infestation did not show significant variation (P>0.05) between age groups, colour as well as systems of production of chicken while there was significant variation (P>0.05) for sex. The infestation with one or more types and species of ectoparasites observed in our study was in tandem with previous studies (Abebe *et al*., 1997 and Belihu *et al*., 2009 in Ethiopia; Swai *et al*., 2010 in Tanzania; Sabuni *et al*., 2010 in Kenya and Nnadi and George (2010) in Nigeria). The different species of ectoparasites identified in this study indicated the existence of diverse ectoparasite fauna in the study area.

The different types and species of ectoparasites as recorded in this study were similar to the studies of Permin *et al*., 2002, Soulsby, 1982, Abebe *et al*., 1997, Sexena *et al*., 1995, Korogu *et al*., 1999.

The overall prevalence of mite infestation was 0% which contradicts the results of other studies. There were reports from some parts of Africa such as in Nigeria by Nnadi and George 2010, in Kenya by Sabuni *et al*., 2010, Yeshitila *et al*., 2011 in Ethiopia and Zumani Banda, 2011 in Malawi where mite infestations occurred in 2.1%, 2.2%, 100% and 1.5% respectively of the studied chickens. The differences observed in the prevalence of mites in these areas might have been associated with poor hygiene in the farms and chicken houses as well as the lack of control measures towards such parasites. In addition, it might also have been due to the type of poultry management systems.

The overall lice infestation in this study (0.32%) was lower than the one reported by Yeshitila *et al*., 2011 in Ethiopia (35.1%), Belihu *et al*., 2009 in Ethiopia (84.3%), Nnadi and George, 2010 in Nigeria (62.2%) and Sabuni *et al*., 2010 in Kenya (14.5%). This could be due to the differences in the practices of ectoparasites control. It could also be due to the time or season of the year when samples were collected. From research (questions), lice infestation is very high at the onset of rainy season but tend to drop drastically when rainfall is fully established. *M. stramineus* was the only prevalent lice species from the study. This finding disagrees with the work of Belihu *et al*., 2009, Zumani Banda 2011, Yeshitila *et al*., 2011 and Sychra *et al*., 2008 who found other species of lice.

The overall prevalence of flea infestation observed in this study (0.53%) was by far less than the report of Belihu *et al*., 2009 in Ethiopia, Swai *et al*., 2010 in Tanzania, Sabuni *et al*., 2010 in Kenya, Yeshitila *et al*., 2011 in Ethiopia and Nnadi and George 2010 in Nigeria who reported 51.2%, 75.3%, 1.5%, 6% and 35.7% respectively. The differences in hygienic and ectoparasite control practices might have played their role in these variations. Loss of chickens to ectoparasite infestation also, has played a role in the variation as many were already lost before the study.

M. domestica (housefly) is considered an ectoparasite of economic and public health importance. Houseflies were the most widespread pests in the study area with a prevalence of 18.01%. Houseflies are least considered as ectoparasites of chickens but obviously are the most common and pose a very big challenge to chickens as they cause noise, disturbance and restlessness to chickens. They also cause worry which brings about decreased egg production in laying birds.

Higher infestation of ticks on chickens (4.24%) may be attributed to their non-predatory attitudes towards the parasites as they were known to feed mostly on seeds and even though they feed on insects they do not feed on ticks. Another hypothetical explanation may be that due to their indiscriminate roaming about for food the ticks could have easy access to them. This finding tends to agree with previous findings from Oluyemi and Roberts (2002) and Biu *et al*. (2007).

Conclusion

Based on this study, it was deduced that ectoparasites of chickens are prevalent in Dutsinma Local Government Area, Katsina State. The study was able to establish significant relationships between host related factors of age and sex and high prevalence of ectoparasites in Dutsinma LGA.

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Conflict of Interests

The authors wish to declare that there is no conflict of interest in relation to this submission.

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EGG PRODUCTION PERFORMANCE OF ISA BROWN LAYER HENS RAISED ON A SMALL SCALE ENTERPRISE IN RURAL NAMIBIA

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Abstract

This study was carried out on a small scale egg production enterprise situated in a rural area of the Zambezi region in Namibia to determine the egg production performance of Isa Brown chickens raised under the deep litter system. Egg production data of 250 chickens in one hen house was collated and analysed. To compare the production of Isa Brown hens against the breed standard and to other commercial breeds, age at 50% production; age at peak production; hen-day egg production (HDEP) and hen-housed egg production (HHEP) parameters at 60, 72 and 80 weeks were computed. A liveability of 91.6% from 18-88 weeks was determined. Fifty percent production was attained at 21 weeks of age and peak egg production at 29 weeks of age at a flock production rate of 87.3%. Hen-housed and hen-day egg production at 60, 72 and 80 weeks were lower than expected for the breed. Average egg production per hen for the laying cycle was 75%. Over the cycle (70 weeks), each hen laid an average of 355 eggs, which translates to about 264 eggs over a period of one year. The hens were culled at a mean weekly production rate of 71%. Overall, the production of layer hens in this study was lower than the breed average, but comparable to the production levels reported in other commercial layer breeds. There is a need to improve the management of hens raised under these conditions in order to maximize on their genetic potential and improve the income of rural farmers.

Key words: egg production, Isa Brown, deep litter, liveability, rural, Namibia

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Introduction

Poultry farming has become a popular farming enterprise that contributes significantly to socio-economic development and human nutrition in almost all the countries around the world (Salam, 2005). According to Pernin and Pedersen (2000), it accounts for more than 30% of all human protein needs worldwide. It is projected that the sector will continue to grow as a result of a shift in consumer preference from red meat to poultry meat and their products (Rosegrant *et al*., 2001) due to health concerns. Eggs and meat are well-documented sources of high quality protein and vitamins and thus contribute to food security. As an enterprise, poultry production supplements revenue from crops and other livestock production enterprises.

The average hen starts laying eggs at between 18 and 20 weeks of age depending on the breed and season (Amin and Hamidi, 2013). After laying eggs for about one year, a hen's egg production, egg shell and content start to decline (Beutler, 2007). Among other factors, egg production varies with breed, geographical location and management system. Reports from around the world indicate that commercial laying chickens can produce 250-320 eggs per year under suitable conditions of management (Grobbelaar, 2008). The Isa Brown is a hybrid hen resulting from crossing the Rhode Island Red and White Leghorn chickens. It is a breed that has become popular in Namibia due to its reported high egg production. These hens have been reported to lay up to 300 eggs per production year (ISA, 2017)

In Namibia, poultry production is still growing, but it has the potential to generate foreign currency earnings through the export of poultry products (NAADS, 2011). One large commercial poultry production enterprise is currently involved in large scale poultry meat and egg production. The rest are small scale egg production businesses serving local communities and towns. The high cost of feed, lack of suppliers of day old chicks and the high mortality associated with young chickens discourage poultry farming (Demeke, 2004).

To date no studies have documented the production performance of layer chickens under rural conditions in Namibia. This study was therefore carried out to assess the egg production performance of Isa Brown hens over a production cycle to determine if the hens are reaching their full production potential in order to draw inferences about the overall management of the flock and make recommendations aimed at improving egg production in the small scale sector under rural conditions.

Materials and Methods

Study area

The study was carried out at an enterprise located in a rural area of Katima Mulilo in the Zambezi region of Namibia. The climate of the region can be broadly categorized into wet (October-April) and dry seasons (May-September). During the wet season, the weather is hot and humid with temperatures of up to 33°C. Rainfall is received from December-March. Little or no rain is received during the dry season which is dominated by cold winter weather.

Management of birds

The layer chicken house had a capacity of 250 hens. The hen house side walls were made of a pole and dagger wall of about 60cm high and wire mesh extending from wall height to roof level. The roof was covered with corrugated zinc sheets. Floors were covered with wood shavings litter and the houses were equipped with nests and perches. Isa Brown pullets were sourced from a reputable commercial breeder at the point of lay (18 months of age). At this stage, the chickens had been vaccinated against the major poultry diseases such as Newcastle Disease and the beaks trimmed. Chickens were exposed to 17.5 hours of both natural and artificial light per day and fed a balanced commercial layer mash twice daily. Water was provided *ad libitum* and changed three times per day.

Data collection

The egg production data of 250 Isa Brown breed hens kept in one hen house that had reached the end of the production cycle was taken with permission of the farm manager. This secondary data comprised of the number of hens per day, daily egg production and the number of eggs collected at different times of the day from the time the hens entered the hen house (June 2015) to the time the hens were considered spent (October 2016). The data was collated, analysed and used to calculate egg production indices for comparison with the breed standard.

Statistical analysis

Data was stored and analysed using simple descriptive statistics in Microsoft Excel ® version 2016. Hen egg production on the farm was assessed and compared to the expected production parameters of the Isa Brown breed of chickens as reported by the breeder using age at 50% rate of lay; age at peak lay; hen-housed egg production (HHEP) at 60, 72, 80 and 88 weeks; hen-day egg production (rate) at peak lay, 60, 72 and 80 weeks and liveability.

Results

Number of hens

Figure 1 shows the number of Isa Brown hens that were in the hen house over the production cycle. The number of hens decreased gradually from 250 at introduction into the hen house to 229 birds at the end of the production cycle. Therefore, 21 birds were culled giving a culling percentage of 8.4% and a liveability of 91.6%.

Egg production

A total of 88,776 eggs were laid over a 70-week period giving an average of 181 eggs per day and an over the cycle hen-day egg production (rate) of 74%. Weekly egg production trends are depicted in Figure 2. The hens entered the laying house at 18 weeks of age. During the first ten days in the hen house, no eggs were laid. Thereafter, egg production rose sharply to reach peak egg production at 29 weeks of age. Egg production fluctuated throughout the production cycle. From 79 weeks of age (61 weeks of lay), egg production started to decline and was lowest at 88 weeks of age (70 weeks into lay) when the hens were culled.

Monthly egg production is depicted in Figure 3. Production rose from the time of introduction of the birds into the house to reach peak production in September 2015. Egg production declined from November 2015 to April 2016, but rose from May-August 2016.

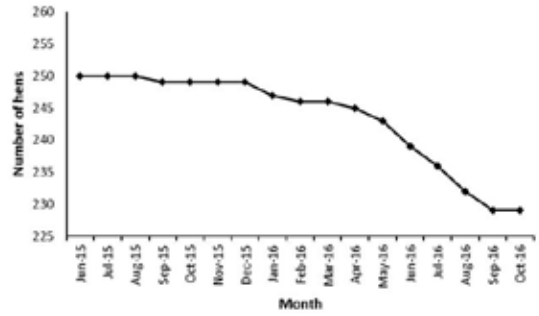

Figure 1: Hen losses during the production cycle

Figure 2: Weekly egg production trends

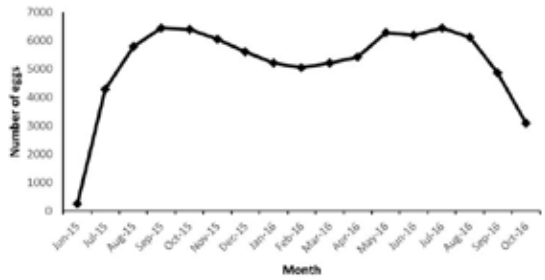

Figure 3: Monthly egg production trends

Egg production indices

Egg production performance indices, age at 50% production; age at peak production; hen-day and hen-housed egg production (rate) at 60, 72 and 80 weeks were computed and are shown in Table 1. Hens reached 50% egg production at 21 weeks of age. Peak egg production was observed at 29 weeks of age when the hens were at a flock production rate of 87.3%. Mean hen egg production was 355 eggs for the cycle, which translates to 264 eggs over a 52-week period.

The largest number of eggs were collected from the hen house in the afternoons (12H00 - 13H00), followed by morning time (06H00) and the least number of eggs was collected in the evenings (18H00).

Table 1: Comparison of egg production indices against the breed standard

Production parameter	Current study	Breed standard[a]
Age at 50% production (weeks)	21	20-21
Age at peak lay (weeks)	29	27-28
Egg production at 60 weeks (HHEP)	203	250
Egg production at 72 weeks (HHEP)	271	313
Egg production at 80 weeks (HHEP)	316	351
Production rate at peak lay (HDEP)	87.3%	95%
Production rate at 60 weeks (HDEP)	69.2%	83.3%
Production rate at 72 weeks (HDEP)	72.2%	75%
Production rate at 80 weeks (HDEP)	74%	68.4%

[a]Egg production indices derived from ISA; HHEP: Hen-housed egg production; DEP: Hen-day egg production

Discussion

Pullets in this study entered the hen laying house at 18 weeks of age but the first eggs were laid at 19 weeks of age, that is, after ten days in the hen house. The age at entering the hen house was within the expected age range of 18-20 weeks for commercial pullets (Beutler, 2007; Yasmeen *et al*., 2008). According to ISA (2017), the ten days prior to the laying of the first egg is a period when the reproductive system is still developing. Hen-housed egg production during the first three weeks of hen house life was one egg per chicken. It takes 2-3 weeks for hens to start laying eggs after entering the hen house (FAO, 2003) and during the first five weeks, egg production is around 10-20% (Meunier and Latour, 2017). The period of low egg production was presumably a period of acclimatization to new feed and the environment.

At the beginning of the study, 250 pullets were placed in the hen house, but 21 were culled over the production cycle giving a liveability of 91.6%, which is less than the breed average of 93.2% (ISA, 2017), but higher than a liveability of 90.57% reported in Isa Brown chickens raised on deep litter (Gerzilov, 2012). Due to the absence of records and post-mortem examination of culled chickens, reasons for culling hens were not available. However, hen mortality and losses vary with the management system and breed of hen. The culling rate of 8.4% reported in this study was comparable to rates of 1-19.9% that have been reported for Isa Brown hens under different management systems (HSUS, 2010). The high egg laying brown chickens including Isa Brown hens are associated with higher mortality rate in the hen house (Häne *et al*., 2000; Berg, 2001; Blockhuis *et al*., 2007).

As expected, the pattern of egg production showed a delay during the first ten days in the hen house followed by a sharp rise to reach and maintain peak production at 29 weeks of age, a month later than the age recommended by ISA (2017). Hens reached 50% production at 21 weeks age as

recommended by the breeder. Towards the end of the production cycle, egg production declined and the hens were culled. In a study by Gerzilov *et al* (2012), Isa Brown hens reared under a similar management system reached peak production late at 35 weeks of age, but at a higher production rate (94.5%) than the 87.3% reported in this study.

The average hen egg production at 60, 72 and 80 weeks recorded in this study was lower than the breed average as reported by ISA (2017) by between 11.1-23.2% indicating that the hens in this study generally had a lower egg production than recommended by the breeder. The average production rate of 75% over the cycle is lower than the 84.6% (Sorensen and Kjaer, 2000) and 76.22% (Fiks-van Niekerk and Reuvekamp, 2009) reported in other studies. Over the entire production cycle, each hen produced an average of 355 eggs, which translates to 264 eggs over a 52-week period. This level of production is lower than the 297 eggs per year reported for Isa Brown hens reared on deep litter by Gerzilov *et al* (2012) in Bulgaria, the 269-286 eggs per year reported in Leghorn hens (Demeke, 2004) and the 420 eggs reported for this breed over a 72 week laying period (ISA, 2017). It was within the range of 250-320 eggs per year reported for commercial chickens by Grobbelaar (2008). The hens in this study produced on average more eggs than the Hyline Brown breed hen which has been reported to lay about 320 eggs over a 74-week period. There was a clear drop in egg production during the summer months of November 2015-April 2016 and a visible rise in egg production in the cooler months of the year (May-August). These findings suggest that weather conditions in this study may have influenced egg production and are in agreement with the findings of Smith and Leclecq (1990) and Oluyemi and Roberts (2000).

The hens were considered spent after 70 weeks of egg laying, although their rate of production of 71% at week 88 was relatively high compared to the rate of 68.4% (ISA, 2017) recommended at the time of culling for this breed. Many factors including egg quality (egg shell and content quality) influence the time of culling and their role in this study could not be confirmed due to the absence of records. However, the overall rate of egg laying had declined and was at its lowest at the time of culling as has been reported by Yasmeen *et al* (2008). A decline in egg production and quality is one of the main reasons given for culling commercial laying hens (Altahat *et al*., 2012). It was noted that the hens had a longer egg production cycle than the 52-54 weeks that is commonly reported for laying hens (Amin and Hamidi, 2013). Further studies are required to assess economic viability of a longer egg laying cycle.

In this study, the highest number of eggs were collected in the afternoons, followed by mornings and the least number of eggs were collected in the evenings. It can therefore be concluded that most hens laid eggs in the morning between 06H00 and 12H00, followed by evening time (18H00- 06H00) and the least number of eggs was laid in the afternoons. These results suggest that the Isa Brown hens laid most of their eggs during the cooler parts of the day. Other studies have reported that hens lay more and more eggs towards the later part of the day as they age (Zakaria *et al*., 2005), but this was not the case in this study.

Conclusions

Hen egg production in this study was lower than has been reported for the Isa Brown breed during one cycle, but was comparable to the production of most commercial layer breeds raised on high technology farms. Hot summer weather negatively affected egg production. There is an opportunity to improve the egg production of Isa Brown hens in this enterprise because the breed is genetically bred to produce more eggs than were recorded in this study. In order to improve egg production, farm income and ensure sustainability, it is recommended that the enterprise identify and correct deficiencies in hen management and keep appropriate records especially those relating to hen mortalities and egg quality.

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SEROLOGICAL SURVEY AND ASSOCIATED RISK FACTORS OF *BRUCELLOSIS* IN PIGS IN CAMEROON

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Abstract

Brucellosis is an important notifiable disease of man and animals worldwide. The epidemiology and risk factors for the disease in cattle are better understood than for other animals in many developing countries wheresurveillance and control activities are biased towards *Bovine* and human *Brucellosis*. In Cameroon, *Bovine Brucellosis* is known to be widespread but the epidemiological situation of *Porcine Brucellosis* is not known. This study was therefore carried out to determine the seroprevalence of *Porcine Brucellosis* in Cameroon and its zoonotic potential among vulnerable communities and populations at risk. The diagnosis was carried out using the Rose Bengal Plate test (RBPT) and indirect ELISA (i-ELISA) while questionnaires completed by pig professionals were used to evaluate the risk factors for *Porcine Brucellosis*. The *Bayesian* approach was used to evaluate the diagnostic tests' sensitivity and specificity. The results showed that of 1081 pigs, 2 (0.19% [0 – 0.45]) were antibrucella seropositive using RBPT and 20 (1.85 % [1.05 –2.65%]) using i-ELISA. *Bayesian* analysis revealed a true prevalence of 3.35% (0.29 – 9.60); sensitivity of 63.8% (50.6 – 79.1) and 82.1% (67.0 – 96.3) and specificity of 99.8% (94.7 – 100) and 98.2% (97.3 – 99.0) for RBPT and i-ELISA, respectively. Breed and age had significant effects ($P< 0.05$) on seropositivity of *Brucellosis* in pigs. All indigenous pigs were seronegative while seropositivity was higher in Duroc hybrids ($P<0.05$) and in pigs aged 12 months or more ($P>0.05$) compared to the other improved breeds and <12 months old pigs, respectively. Factors such as the common use of reproductive boars for breeding, frequent problems of reproduction disorders in pig farms as well as ignorance of the hazards and modes of transmission of zoonotic *Brucellosis* by pig professionals showed increased potential for *Brucellosis* in pigs and the exposure of pig professionals to infection. This study reports the first evidence of anti-brucella porcine seropositivity in Cameroon and revealed that *Brucellosis* is a real pig and human health problem in piggery structures in the country. Public awareness campaigns and health education especially among livestock professionals and in agropastoral communities should be highlighted to disseminate knowledge on the potential risk factors and protective measures against zoonotic *Brucellosis*. The sensitization of animal professionals to improve their level of awareness and intensification of the integrated "One Health" approach for the effective management of zoonoses in Cameroon should be emphasized.

Key words: *Porcine Brucellosis*, seroprevalence, risk factors, *Bayesian* analysis, Cameroon

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Introduction

The demand for animal protein in Cameroon is high and increasing. There are efforts to promote and improve the husbandry of animal species with short reproduction cycles including pigs (Akoa 2006; Keambou *et al*. 2010). However, pig production in the country is constrained by many factors including organization, genetics, feeding, health and disease (MINEPIA 2009; Ndebi *et al*. 2009; Tankou 2014). Although African Swine fever is the most important pig disease (MINEPIA 2009), there are many devastating and neglected diseases that affect pig health and production in Cameroon. Indeed, several pig abortion cases have been reported (Tuékam 1983) which were linked to *Porcine Brucellosis* (Ogundipe *et al*. 2001; Mangen *et al*. 2002; Moussa *et al*. 2013).

Porcine Brucellosis is caused by Brucella suis and occasionally by Brucella abortus and Brucella melitensis (Algers *et al*. 2009; OIE 2012). It has been associated with huge economic losses due to factors in sows related to return to heat after insemination, decreased farrowing percentage and litter sizes, delayed farrowing, culling for infertility, cost of treatment, abortions, stillbirths, birth of mixed live but weak and dead piglets, orchitis in boars and loss of man-hours in infected communities (Ogundipe *et al*. 2001; Mangen *et al*. 2002; Cventnic *et al*. 2004; Algers *et al*. 2009; CFSPH 2009; Cvetnic *et al*. 2009; Mai *et al*. 2012; OIE 2012; Diaz 2013; Moussa *et al*. 2013; Poester *et al*. 2013). Non-specific symptoms have been reported such as arthritis in males and females as well as orchitis and infection of accessory sexual organs in males. Zoonotic *Brucellosis* also causes reproductive, nervous and other clinical disorders in humans (Chakroun & Bouzouaia 2007; Mohamed *et al*. 2010), who are usually occupationally exposed to infected pigs (OIE 2012). Human *Brucellosis* has often been misdiagnosed and mistreated as other debilitating diseases such as malaria and typhoid fever (Racloz *et al*. 2013; Njeru *et al*. 2016).

Brucellosis is an important disease among livestock and people in sub-Saharan Africa where the prevalence of risk factors for infections are better understood for *Bovine Brucellosis* and to a lesser extent for ovine, caprine and human *Brucellosis* (McDermott & Arimi 2002; Akakpo *et al*. 2009; Ducrotoy *et al*. 2014; Ducrotoy *et al*. 2017). The occurrence and epidemiology of *Brucellosis* in pigs worldwide is poorly understood and not investigated (McDermott & Arimi 2002; Njeru *et al*. 2016; Ducrotoy *et al*. 2017). Although this species bias is also reflected in surveillance and control activities of animal and human *Brucellosis* (Boukary *et al*. 2014; Ducrotoy *et al*. 2017), the transmission of *Brucellosis* in pigs is similar to those identified for *Brucellosis* in other animal types; being essentially through the oral, nasopharyngeal, conjunctival and vaginal mucosae (Algers *et al*. 2009). There is *generally* a relatively long incubation period before clinical signs appear, it is not usually visible in young animals, and its occurrence will depend mainly on the age, sex and physiological state of the animals at the time they are infected (Enright 1990). Infected pigs excrete Brucellas in urine, sperm, vaginal discharge, milk, and also by the placenta, lochial secretions, aborted fetuses and the content of subcutaneous brucellous abscesses (MacMillan 1999; Algers *et al*. 2009). *Brucellosis* seroprevalence has been reported in some African countries including Tchad (4%), Guinea (30%) and Garbon (0%) (Akakpo & Ndour 2013), Ethiopia (4.5%) (Kebeta *et al*. 2015), Uganda (<1%) (Erume *et al*. 2016), Kenya (0.2%) (Njeru *et al*. 2016) and abattoirs in the Northcentral (30.6%) (Ngbede *et al*. 2013); Southwest (0%) (Cadmus *et al*. 2006) and Southeast (0.6%) (Onunkwo *et al*. 2011) of Nigeria. However, there is a dearth of information on the epidemiological situation of *Porcine Brucellosis* in Cameroon where *Bovine Brucellosis* is widely endemic and seroprevalence rates ranging from 3 – 16% at individual cattle level and 16.2 – 35.0 % at herd levels have been reported (Shey-Njila *et al*. 2005a; Shey-Njila *et al*. 2005b; Bayemi *et al*. 2009; Scolamacchia *et al*. 2010; Mazeri *et al*. 2013; Bayemi *et al*. 2015; Ojong 2015; Awah-Ndukum *et al*. 2018). There are also uncontrolled livestock movements across national and international borders and mixed animal husbandry practices are

common in the country (Awah-Ndukum *et al.* 2014). The occurrence of the disease in other farm animals such as sheep, goats and horses are poorly understood and determining the prevalence and risk factors of *Brucellosis* in all livestock according to their origin would improve understanding of the epidemiology of the disease in Cameroon.

Although *Brucellosis* is an important notifiable disease in man and animals worldwide, there is no information on the zoonotic potential and occurrence of *Porcine Brucellosis* in Cameroon particularly among vulnerable communities and populations at risk including livestock and livestock professionals. Also, potential factors for the occurrence and spread of *Porcine Brucellosis* such as infected domestic ruminants and wild animals sharing the same micro-environment as domestic pigs (Algers *et al.* 2009; CFSPH 2009; MINEPIA 2009; Ndebi *et al.* 2009; Bronner & Garin-bastuji 2010; Kaoud *et al.* 2010; Marce & Garin-bastuji 2011; Njeru *et al.* 2016) occur in Cameroon. It is in this context that this study was carried out to contribute to the knowledge on the epidemiology of *Porcine Brucellosis* in Cameroon through the determination of the seroprevalence distribution and risk factors of *Brucellosis* in pigs in Cameroon.

Materials and methods

Description of study areas

This study was carried out during the period of August 2015 and January 2016 in pig farms of three administrative divisions (Diamaré, Mayo-Danay, Mayo-Kani) of the Far-North region (10° – 13° LN and 13° – 15° LE) and the central pig market and slaughter area of Douala II in Wouri administrative divisions (4°20° – 4°35° LN and 9°35° – 9°50° LE) in the Littoral region of Cameroon (Figure 1).

The population of the Far-North regions is essentially rural (77.3%) and the principal economic activities are agriculture, animal husbandry, fishing and commerce (Houwe 2011; MINEPAT 2014). The climate is the Sudano-Sahelian tropical type characterized by two seasons (long dry season from October to April and short rainy season from May to September), 600 – 1000 mm rainfall per annum and 20°C – 40°C environmental temperature (MINEPAT 2014). Douala has a cosmopolitan population (62.4% urban), equatorial type climate characterized by four seasons (2 rainy and 2 dry seasons), average of 4000 mm rainfall per annum, relative humidity of about 85% and poor sunlight (MINEPAT 2010).

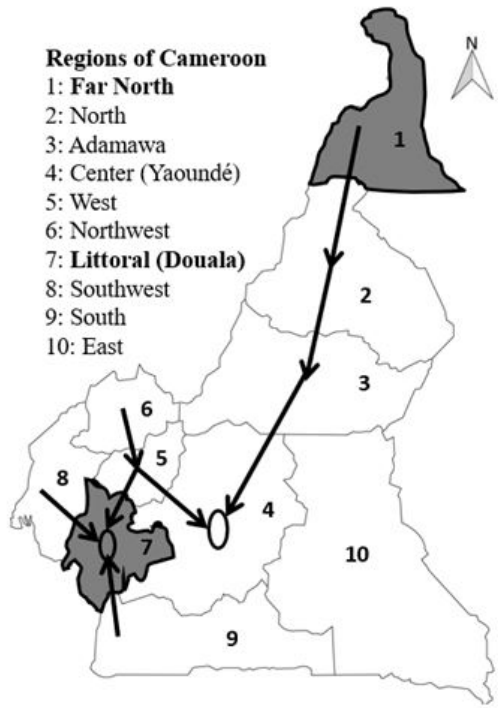

Figure 1: Map showing the study regions (Far-North and Littoral Regions) and major commercialization corridors (arrows) and markets (Yaoundé and Douala) of pigs in Cameroon

The pig husbandry is very widespread in Cameroon with the Far North, Northwest, West and Littoral regions being the major pig producing areas and the other regions (Adamawa, Centre, East, North, South and Southwest) ranging from small to moderate producers (MINEPIA 2009). The commercialization of pig and pig products in Cameroon (Figure 1) shows that consumption areas are furnished by multitudes of pig producers spread throughout the country (Ndebi & Ongla 2006); with the biggest pig

markets being in Yaoundé (Centre Region) and Douala (Littoral Region) as well as other markets in the Far-North and West Regions. Also, it is common to observe important influxes of pigs from Southwest Chad through Mayo-Danay and Mayo-Kani divisions of the Far-North regions destined for the markets in Yaoundé (MINEPIA 2009). Before the 2010 epidemic outbreak of African Swine Fever in the Northern regions of Cameroon (MINEPIA 2011), pig and pig products sold and consumed in Yaoundé originate mainly from the Adamawa, North and Far-North regions (over 58%) as well as from the West and Northwest regions (about 42%). While the market in Douala is mainly supplied by the West and Northwest regions (Ndebi & Ongla 2006; MINEPIA 2011).

Selection of study animals

Pig farms: Pig farms in three administrative divisions (Diamaré, Mayo-Danay, Mayo-Kani) of the Far-North region were sampled for the study. For lack of previously reported prevalence, a default rate of 50% was used to estimate the number of pigs required to detect ≥1 infected animal with a desired 95% confidence and precision of ≥ 5% as previously described (Thrusfield 2007). The selection of pig herds was done by the random-number *generat*ion method of pig keeping communities, pig owners and locations of pig farms from records at the Divisional Delegations of Livestock, Fishery and Animal Industries (DDEPIA). The herd sizes of the chosen pig farms ranged from 5 to 50 pigs and 30 – 60 % of animals within the selected farms depending on larger (≥ 30%) to smaller (≤ 60%) sampling rate per herd were done, respectively. Weaned piglets and pigs less than six months, due to poor reactions to various serological tests for *Brucellosis* (Kaoud *et al*. 2010; Marce & Garin-bastuji 2011; OIE 2012), fattening pigs (pigs being fattened and not intended for breeding) and pigs that could not be humanely captured and restrained were excluded from the study. Information relating to location, husbandry practices, breed, physiological status, sex and age of the animals obtained from farm records as previously described (CDDR/SAID 1996; FAO 2009; Houwe 2011; AU-IBAR 2015; Kouamo *et al*. 2015) was noted. The animals in this study were reared traditionally with or without shelter in free range (scavenging) and semi-intensive systems. They were composed of the indigenous pigs and various hybrids (improved breeds).

The Douala II central pig market and slaughter area: The pig slaughter area and central pig market of Douala II in Wouri Division of Littoral region are adjacent to each other. Usually, less than 10% of the pigs destined for human consumption are subjected to routine veterinary inspections in most developing countries (FAO 2013) including Cameroon. The Douala pig market and slaughter areas are typically chaotic and routine ante and post-mortem inspection of pigs is not done. Slaughtered adult pigs in Douala II were sampled for the study and the selection of pigs for the study was based on random arrival of animals for slaughter. Young pigs less than six months as well as fattened pigs that have never bred were excluded from the study. Briefly, about 20% of 40 – 60 pigs slaughtered daily in the Douala slaughter area was randomly selected each day, except on Saturday and Sunday, and included in the study. Based on a calculated sampling fraction of five (every fifth animal was sampled) for daily use, the first animal was selected by picking an animal by the random *generat*ion method from the first five animals on the slaughter chain. Thereafter, every fifth animal (adding 5 to the previously picked number) was chosen and sampled. Information related to the sex, age, physiological status and sexual maturity using morphological appreciations (CDDR/SAID 1996; FAO 2009; Houwe 2011; AU-IBAR 2015; Kouamo *et al*. 2015) as well as previous functions of the animals, were obtained from the veterinary health certificates, pig professionals and as previously described (CDDR/SAID 1996; Houwe 2011) and noted. The breeds involved were previously described (FAO 2009; AU-IBAR 2015; Kouamo *et al*. 2015). The pigs slaughtered in Douala during the study period originated from Littoral, Northwest, West and South regions of the country.

Overall, a total 1,081 pigs from herds in Diamaré (183), Mayo-Danay (117) and Mayo-Kani (156) in the Far-North region (456) and pigs of the Douala II central pig market and slaughtering area (625) in the Littoral region were sampled for the study. They were composed of 443 indigenous pigs and 638 improved breeds which were products of multiple crossings between local breeds and exotic breeds (66 Berkshire hybrids, 60 Duroc hybrids, 225 Landrace hybrids, 28 Large white hybrids and 259 Pietrain hybrids), following successive imports and supplies of exotic parents.

Serological Tests

Blood (≥5ml) was collected through jugular venipuncture of the pigs in the study using sterile vacutainer tubes for blood collection, serum extraction and storage at –20°C until laboratory analysis at the Veterinary Research Laboratory of IRAD, Wakwa Regional Center, Ngaoundéré, Cameroon. Rose Bengal Plate test (RBPT) and indirect Enzyme Linked Immunosorbent Assay (i-ELISA) Test were performed on the serum samples.

Rose Bengal Plate Test: RBPT was performed as described by Alton *et al*. (1988). Briefly, the sera and antigen were brought to room temperature before use. Equal volumes (30 μL) of standardized B. abortus antigen Weybridge strain 99 and test serum were mixed thoroughly and rotated on a glass plate using a stick applicator and the plate was rocked for 4 minutes. The appearance of agglutination, recorded as positive, within 1 minute was scored 4+ (++++) and between 1 and 4 minutes was scored 1+ to 3+ (+, + +, and + + +) according to the different degrees of agglutination. The absence of agglutination within 4 minutes was regarded as negative (-).

Indirect enzyme-linked immunosorbent assay: i-ELISA (ID.Vet, Innovative Diagnostics, France) was performed according to the manufacturer's instructions and essentially as described by Limet *et al*. (1988). The test was conducted in 96-well polystyrene plates that were pre-coated with purified Brucella abortus lipopolysaccharide (LPS) antigen. A multispecies horseradish peroxidase (HRP) was used as conjugate as described by Saegerman *et al*. (2004). The substrate solution (TMB + DMSO + H2O2) was added after washing to eliminate excess conjugate. The coloration of antigen-antibody conjugate-peroxidase complex formed depended on the quantity of anti-Brucella antibodies that was present in the specimen tested. Thus, in the presence of antibodies, a blue solution appeared which became yellow after addition of the Stop Solution, while in the absence of antibodies, no coloration appeared. The microplate was read at 450 nm using an automatic ELISA reader and for each sample S/P% was calculated as follows: $S/P\% = (OD_{sample} - OD_{nc})/(OD_{pc} - OD_{nc}) \times 100$ where ODsample, ODnc and ODpc were the reading of optical densities for the sample, negative control and positive control, respectively. The samples were classified as positive if S/P % ≥ 120 %, negative if S/P % ≤ 110 % and doubtful if 110 % < S/P % < 120 %. Also, $OD_{pc} > 0.350$ and $OD_{pc}/OD_{nc} > 3$, indicated that the test was working properly.

Risk factor analysis

Information on risk factors for *Porcine Brucellosis* was obtained by questionnaire interviews of pig professionals / handlers (pig farmers and traders, butchers and butcher-aids; transporters of live pigs, pork and pig carcasses; slaughterhouse administrative staff and pork roasters) in the Douala II central pig market and slaughter area. The questionnaires were semi-structured to collect information on a range of variables including animal management and husbandry practices, demographic information, and levels of awareness and human exposure to zoonotic *Porcine Brucellosis*. Veterinary inspectors at the study sites were not included in the survey since they are knowledgeable about zoonotic *Brucellosis* (MINEPIA, personal communication).

Ethical consideration

Risk assessments of the project were performed by the researchers to avoid hazards to all persons and animals involved in the project. Safe procedural restraining

manipulations were used and the animals were not subjected to suffering. Ethical clearances were obtained from the required authorities (MINEPIA Delegations in Far-North and Littoral regions, School of Veterinary Medicine and Sciences/University of Ngaoundéré) before carrying out the study. The purpose of the study was explained to the pig professionals with the assistance of local veterinarians, community leaders and trusted intermediaries. Pig farms and pigs destined for slaughter were sampled and interview questionnaire surveys were done after informed consents were given by the animal owners.

Data analysis

The data were analysed using "R" software. Simple percentages were *generated* and the Chi-square test was used to assess the association between factors and the odds-ratios determined for associated risk factors along 95% confidence intervals and statistical significance set at $P<0.05$. The *Bayesian* approach was used to evaluate the diagnostic tests' sensitivity and specificity and estimate the true prevalence, based on conditional dependence between the tests in the absence of a gold standard method (Berkvens *et al.* 2006; Praet *et al.* 2006; Sanogo *et al.* 2008; Sanogo *et al.* 2013). The sensitivity and specificity of the two tests were evaluated by subjecting each sample to the two tests and the observed data of the two tests summarized in cross tabulation.

Results

Seroprevalence of Porcine Brucellosis and characteristics of the diagnostic tests

The study showed that of 1,081 pigs, 2 (0.19% [0 – 0.45]) were anti-brucella seropositive when tested using RBPT and 20 (1.85 % [1.05 –2.65%]) using the i-ELISA (Table 1). The combination results of both tests in the *Bayesian* model revealed a true prevalence of 3.35% (0.29 – 9.60). The test characteristics of 63.8% (50.6 – 79.1) and 82.1% (67.0 – 96.3) as sensitivity and 99.8% (94.7 – 100) and 98.2% (97.3 – 99.0) as specificity for RBPT and i-ELISA, respectively, were determined after combining the results with expert's opinion on the *Bayesian* model. The validation of the model used to determine the true prevalence and tests' characteristics are summarised in Table 2.

Risk factors of Porcine Brucellosis in Cameroon

Factors affecting seroprevalence of *Porcine Brucellosis*: The study revealed that breed and age had significant effects ($P< 0.05$) on the seropositivity for *Brucellosis* in pigs (Table 3). although all indigenous pigs

Table 1: Combined results of Rose Bengal Plate test and indirect enzyme linked immunosorbent assay among pigs in Cameroon (n=1081)

Serological results	Number of cases (% [95% CI])
RBPT (+)	2 (0.19% [0 – 0.45])
i-ELISA (+)	20 (1.85% [1.05 – 2.65])
RBPT (+) i-ELISA (+)	2 (0.19% [0 – 0.45])
RBPT (+) i-ELISA (-)	0 (0%)
RBPT (-) i-ELISA (+)	18 (1.67% [0.91 – 2.43])

(□): negative; (+): positive; i-ELISA: indirect Enzyme-Linked Immunosorbent Assay; RBPT: Rose Bengal Plate test.

Table 2: Validation of *Bayesian* model used to estimate the true prevalence of *Porcine Brucellosis* in Cameroon and the sensitivity and specificity of RBPT and i-ELISA.

Combined test	Bayes p-value	PD_Pr	PD_p	DIC_Pr	DIC_P
Ag-ELISA/Ab-ELISA	0.589	1.654	13.265	13.359	36.378

Bayesp: Bayesian-p value; DIC: Deviance Information Criterion; PD: The effective number of parameters; Pr: Multinomial probabilities; P : Parameters in the model using parent nodes; DIC_Pr: DIC values from posterior mean of the multinomial probabilities; DIC_P: DIC values from posterior mean of the parameters in the model using parent nodes

(443) as well as pigs from the Far North (456) and South (24) regions were *Brucellosis* seronegative and there was no difference (P>0.05) in the seroprevalence recorded in the southern regions between origin of the animals, pigs from the Northwest region showed the highest seroprevalence followed by the Littoral and West regions. Overall, there was no difference (P>0.05) between sex and husbandry systems. *Brucellosis* seroprevalence was significantly higher particularly in the Duroc hybrids (P<0.05), 12 – 24 months old pigs [OR= 3.72 (1.21 – 11.51); P<0.05] and > 24 months old pigs [OR = 1.97 (0.44 – 8.91); P>0.05] compared to the other improved pig breeds and <12 months old pigs, respectively.

Potential risks of human exposure to zoonotic *Porcine Brucellosis*: Overall, 93.23% (124 of 133) of pig professionals / handlers in the Douala pig market / slaughter area responded to the questionnaire including pig breeders and traders (20), slaughtering butchers (28), butcher aides / apprentices (46), pork roasters (20), transporters of live pigs and pig carcasses / pork (3) and administrative staff (7). The respondents were composed of 106 males and 18 females, predominantly 30 – 50 years (81), married (96) and with a post primary educational status (78).

The potential risk factors associated with the exposure of pig professionals / handlers (n=124) to zoonotic *Porcine Brucellosis* are summarized in Figure 2. Although over 84.68% (105) of pig professionals undertook measures (particularly good personal hygiene, wearing protective clothes and footwears) that are protective against contracting *Brucellosis* during work, most were ignorant of the hazards (> 94.5%) and modes of transmission (> 98.5%) of *Brucellosis* in animals (including pigs) and humans. Among 91.94% (114) of respondents who were not equipped with first-aid kits at

Table 3: Risk factor model for *Brucellosis* seropositivity in individual pigs (n=1081) in Cameroon

Category	Variable	Number (Positive)	Seropositivity using i-ELISA: % [95%CI]	P-value (χ^2)
Total		1081 (20)	1.85 [1.05 - 2.65]	0.1571 (3.7013)
Region of origin	Littoral	109 (4)	3.67 [0.14 – 7.20]	
	Northwest	116 (7)	6.03 [1.70 – 10.37]	
	West	376 (9)	2.39 [0.85 – 3.94]	
	South	24 (0)	0	
	Far North	456 (0)	0	
Sex	Female	734 (15)	2.04 [1.02 – 3.07]	0.4924 (0.4713)
	Male	347 (5)	1.44 [0.19 – 2.69]	
Age (months)	Young (6 – 12)	475 (4)	0.84 [0.02 – 1.66]	0.0462* (6.1507)
	Adult (12 –24)	424 (13)	1.85 [1.05 – 2.65]	
	Old (>24)	182 (3)	3.67 [0.14 – 7.20]	
Improved breeds	Berkshire hybrid	66 (1)	1.52 [0 – 4.48]	0.0251* (11.138)
	Duroc hybrid	60 (6)	10.00 [2.41 – 17.59]	
	Landrace hybrid	225 (7)	3.11 [0.84 – 5.38]	
	Largewhite hybrid	28 (1)	3.57 [0 – 10.44]	
	Pietrain hybrid	259 (5)	1.93 [0.25 – 3.61]	
	Indigenous pigs	443 (0)	0	
Husbandry system	Extensive	730 (12)	1.64 [0.72 – 2.56]	0.4679 (0.5269)
	Semi-intensive	351 (8)	2.28 [0.72 – 3.84]	

**Significantly different (P<0.05).*

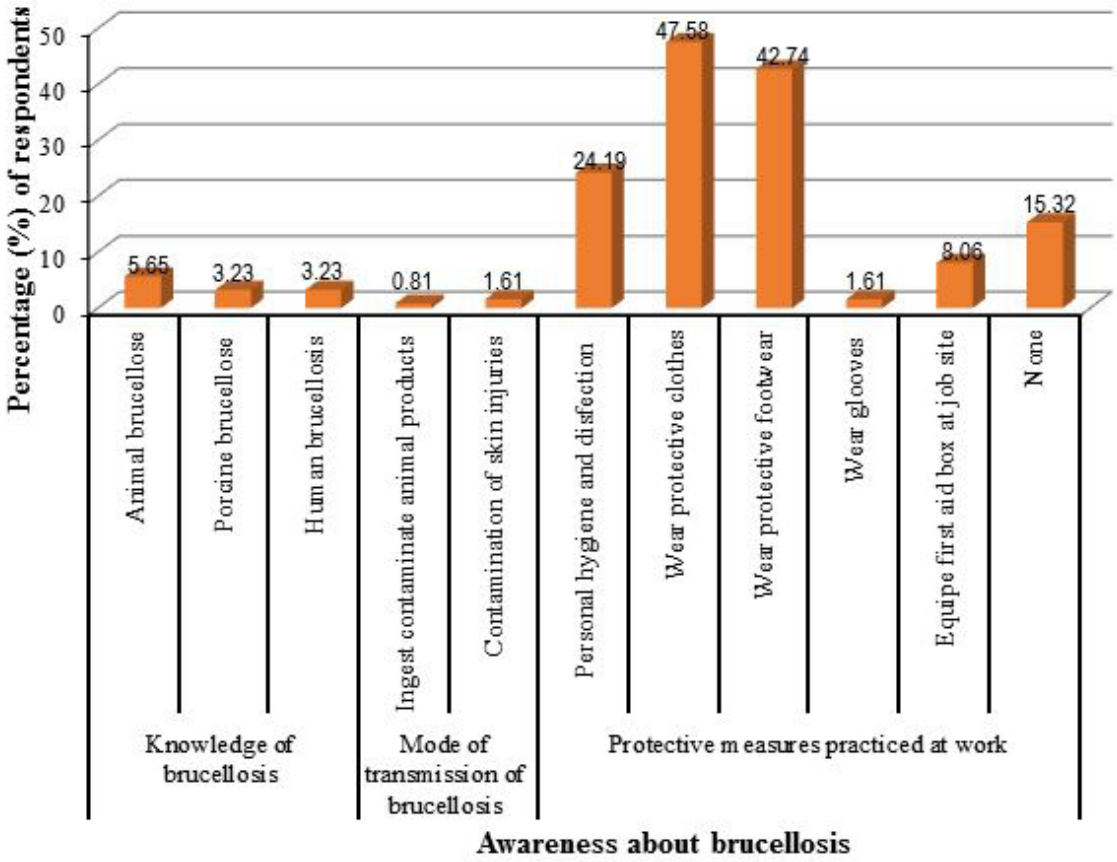

Figure 2: Degree of awareness about *Brucellosis* among pig professionals in the Douala II pig market and slaughter area

work, over 57.89% (66) of them rushed to near-by health facilities and 14.91% (17) used unconventional therapeutic methods in the event of injurious accidents during work; while the remaining 27.19% (31) did not respond.

The respondents in study also revealed other factors which could influence *Porcine Brucellosis* in pig farms and increase exposure of pig handlers to *Porcine Brucellosis* such as sharing, renting and hiring of reproductive boars for breeding [25% (31)] and frequent problems of reproduction in sows [47.58% (59)]; namely stillbirths [33 (55.93%)], abortion [21 (35.59%)] and farrowing of puny (small and weak) piglets [5 (8.47%)]. Furthermore, married respondents (n=96) who had handled pigs for at least 5 years reported reproductive disorders in their family including miscarriages (14.58%), stillbirths (11.46%) and infertility (20.83%).

Discussion

Although pig husbandry is widespread in Cameroon and many communities depend largely on pigs for their livelihoods, there is little or no attention on the occurrence and prevalence of *Brucellosis* in pigs. This study presents the first report of anti-brucella seropositivity in pigs in Cameroon that also determined the *Porcine Brucellosis* seroprevalence of over 3.35% in the country based on the field data of pigs originating from five regions (Littoral, Northwest, West, South and Far North) and combination results of RBPT and i-ELISA tests with expert's opinion in the *Bayesian* model. Sensitivity (63.8% for RBPT and 82.1% for i-ELISA) and specificity (99.8%

for RBPT and 98.2% for i-ELISA) values were also determined. The deviance information criterion (DIC) combines a *Bayesian* measure of fit with a measure of model complexity and is the most popular criterion for *Bayesian* model selection and model comparison (Shriner & Yi 2009). Low DIC values for the *Bayesian* models, indicating good fit of the models (Francois & Laval 2011), and high *Bayesian* p-values (Marsman & Wagenmakers 2016) obtained in this study supported the test characteristics described for RBPT and i-ELISA. *Bayesian* analysis of serological tests including RBPT and i-ELISA, using field sera, for the diagnosis of *Porcine Brucellosis* have recorded similar sensitivity (≈80%) and specificity (≈100%) results (Algers *et al*. 2009; Muñoz *et al*. 2012; Praud *et al*. 2012). The results of this study suggest that *Porcine Brucellosis* is a real public health threat in Cameroon, that is not investigated, and infected animals through their excretions (MacMillan 1999; Algers *et al*. 2009), can serve as reservoirs and sources for human *Brucellosis* in the regions. Human *Brucellosis* may be present in the country among pig farmers and professionals and also among people who live in pig rearing communities, habitually consuming poorly cooked and raw pork as well as handling and trading in pigs and pig products.

The study observed that age and breed were major factors for high porcine brucella seropositivity compared to sex and husbandry system that had no significant influence on the seroprevalence. The study showed that adult and old (≥12 months) pigs had increased odds of being seropositive reactors than younger pigs. This agrees with previous reports which explained that sexually mature animals are more susceptible (Kouamo *et al*. 2010) and older animal are potentially exposed longer to the disease than younger animals (Mazeri *et al*. 2013; Awah-Ndukum *et al*. 2018). The finding has some similarity with previous findings by Diaz (2013), who reported higher seropositivity among improved breeds (Pietrain, Landrace and Duroc hybrids) but did not observe differences in seropositivity due to age. On the contrary, Ngbede *et al* (2013) found relatively higher sero-prevalence in male than female pigs in Nigeria while Kebeta *et al* (2015) recorded higher seroprevalence in female than male pigs in Ethiopia and associated the difference to the fact that the female reproductive tract is a potential reservoir for Brucella spp to propagate. The exclusion of weaned piglets and pigs less than six months, due to poor reactions to various serological tests for *Brucellosis* (Kaoud *et al*. 2010; Marce & Garin-bastuji 2011; OIE 2012) as well as the higher exploitation of young animals and other management practices in farms might have played additional roles in the different seropositivity results due to breed and age in the study.

Pigs sampled in the more arid Northern regions were seronegative compared to the occurrence of seropositive pigs in the more humid southern regions. Although the highest sero-prevalence rates were recorded in the Northwest region followed by the Littoral and West regions, the origin of animal did not significantly influence the seroprevalence rates within the southern regions. This finding agrees with Kebeta *et al* (2015) who reported that there was significant difference in porcine seroprevalence between sex and origin of the animals; with a higher prevalence found in humid climatic environments. Many previous studies (Domenech *et al*. 1982; Akakpo 1987; Akakpo & Bornarel 1987; Sanogo *et al*. 2013; Boukary *et al*. 2014; Awah-Ndukum *et al*. 2018) have reported that transmission of *Bovine Brucellosis* increases from arid to humid regions and higher *Bovine Brucellosis* seroprevalence rates occur in typical tropical ambient humid (Guinean or Savannah Guinean zones) climate than in tropical hot dry (Sudano or Sudano-Sahelian zones) climate. The average humidity (<40%) and rainfall per annum (600 – 1000 mm) are lower while the sunlight and environmental temperatures (20°C – 40°C) are higher in the Far North region compared to higher humidity (85%) and rainfall per annum (2000 – 4000 mm) and poor sunlight and lower environmental temperatures (15°C – 25°C) in the southern regions (LT, NW, S, W) (MINEPAT 2010; Houwe 2011). Brucella spp is rapidly destroyed by hot and dry climates and survives long periods in fresh pastures and in cool humid conditions

(Akakpo 1987; Akakpo & Bornarel 1987; Corbel 2006; Algers *et al*. 2009).

Bovine Brucellosis is widespread in Cameroon including the regions sampled in this study (Shey-Njila *et al*. 2005b; Bayemi *et al*. 2009; Scolamacchia *et al*. 2010; Mazeri *et al*. 2013; Bayemi *et al*. 2015; Ojong 2015; Awah-Ndukum *et al*. 2018) and the existence of *Bovine Brucellosis* seemed to have favoured the occurrence of *Porcine Brucellosis* in the West, Littoral and Northwest areas in this study. This agrees with earlier reports that *Brucellosis* in pigs may occur in regions where *Brucellosis* is endemic in domestic ruminants (Radostits *et al*. 1995; Young 1995; Onunkwo *et al*. 2011). This is contrary to 0% *Porcine Brucellosis* seroprevalence recorded in the Far North region of this study as well as the findings of Cadmus *et al*., (2006) who reported *Brucellosis* seroprevalence of 0% in pigs and 5.82% in cattle and 0.86% in goats in an abattoir in Nigeria. The pigs unlike the ruminants could have been kept and or originated from areas where livestock *Brucellosis* is not common. Also, there could have been no contact / interaction between pigs and ruminants even if the disease is endemic in the ruminants. In far North Cameroon, cattle keeping is predominantly done by the Muslim and pigs by non-Muslim communities with practically little or no contact between pigs and cattle in the region.

Although the level of susceptibility of breed to *Brucellosis* was not ascertained by the study, suggesting further investigation, the indigenous breeds were anti-brucella seronegative reactors. The indigenous breeds (basically reared traditionally with or without shelter such in free range and scavenging systems) in the study were predominantly in the dryer Far North region and hardier compared to improved breeds (products of multiple crossings between local breeds and exotic breeds, following the successive imports and supplies of exotic parents) that were reared in more confined systems and humid southern regions. This finding suggests that the serological incidence of the disease was lower where extensive systems dominate compared to more intensified systems. Several studies on *Bovine Brucellosis* have recorded the survival of Brucella species for several months in intensive systems with poor hygienic practices (such as the presence of stagnant water, slurry, waste hay and cracks in walls in the farms) (McDermott & Arimi 2002; Boukary *et al*. 2014). Persistent *Brucellosis* and higher seroprevalence among cattle kept in extensive systems and mix livestock (cattle, small ruminants) herds (McDermott & Arimi 2002; Racloz *et al*. 2013; Boukary *et al*. 2014; Njeru *et al*. 2016) and higher rates in hybrid cattle than in the indigenous zebus have been reported (Akakpo 1987; Akakpo & Bornarel 1987; Boukary *et al*. 2014). Although the pattern of antibody production in brucella infected pigs has not been properly established, it should be similar to the case of other brucella infections (Algers *et al*. 2009). Specific IgM anti-brucella antibodies predominate in the first 2 weeks after infection while IgG anti-brucella antibodies increases slowly in the blood over the first 3 weeks of infection (Algers *et al*. 2009; Nielsen & Yu 2010). Animals may lose their anti-brucella antibody titres without seroconversion following mild or latent infections without further exposure to infection and sources of infection (Sippel *et al*. 1982). Nonetheless, *general* poor condition of animals, aging and high parity have been observed to significantly increase *Brucellosis* seroprevelance and animals become more sensitive to brucella infection at the reproductive age (Domenech *et al*. 1980; Akakpo 1987; Akakpo & Bornarel 1987; Kpomassi 1991; Algers *et al*. 2009; Bayemi *et al*. 2009; Kouamo *et al*. 2010; Boukary *et al*. 2011).

In the study about 85% of pig professionals used at least one protective measure (e.g. good personal hygiene, wearing protective clothes and footwear) against contacting *Brucellosis* during work. However, very high levels of ignorant of pig professionals / handlers of the hazards (> 94.5%), risk factors and modes of transmission (> 98.5%) of zoonotic *Brucellosis* in animals and humans was noted. It is worth noting that, *Brucellosis* due to Brucella suis strains of separate origin have been detected among workers at a pig slaughterhouse (with serological evidence

of *Porcine Brucellosis*) who showed signs and symptoms compatible with *Brucellosis* (Escobar *et al*. 2013). Several reports focusing on *Bovine Brucellosis* in Cameroon, Nigeria, Tanzania and Egypt have highlighted higher seroprevalence rates of *Brucellosis* among livestock professionals (e.g. uneducated animal handlers, butchers, herdsmen, livestock owners and traders) less informed about zoonotic *Brucellosis* than in more knowledgeable professionals (e.g. educated animal handlers, veterinarians and para-veterinarians) and persons with short exposure time to animal products and good personal hygiene practices (eg administrative staff, animal owners, traders of animals and animal products who are knowledgeable) (Cadmus *et al*. 2006; El Kholy *et al*. 2009; Swai & Schoonman 2009; Aworh *et al*. 2013). Ignorance of *Porcine Brucellosis*, lack of traceability of pigs destined for slaughter and abattoir environments conducive for the survival of Brucella species which constitute major factors for human exposure to zoonotic infection (Algers *et al*. 2009; Boukary *et al*. 2014; Njeru *et al*. 2016; Awah-Ndukum *et al*. 2018), were noted in this study. Other potential factors associated with human *Brucellosis* seroprevalence in livestock professional groups include consuming raw milk, handling animal fetuses and aborted animals, occupational exposure of over 5 years, little or no knowledge of *Brucellosis*, contact with livestock and religious practices (Cooper 1992; Alballa 1995; Kumar *et al*. 2000; Fatima & Farklanda 2008; Mugabi 2012; 2015; Njeru *et al*. 2016). Similar risk situations have been described among pig professionals / handlers for *Brucellosis* as well as the need to improve anti-*Brucellosis* education and the routine screening of animals for swine *Brucellosis* (Escobar *et al*. 2013).

Several observations of false positive reactions in the serological diagnosis of *Brucellosis* due to close antigenic cross reactivity with other bacterial infections (Yersina, Xanthomous, Salmonella, Streptococci, *E. coli*, Tuberculosis) have been reported (Pouillot *et al*. 1998; Yildiz *et al*. 2005; Sanogo *et al*. 2008; Varshochi *et al*. 2011; Szulowski *et al*. 2013). Although the association between false positive serological reactions and *Bovine Brucellosis* is not clear (Pouillot *et al*. 1998), false positive serological reactions in *Porcine Brucellosis* serology are common (OIE 2012; Szulowski *et al*. 2013; Weiner *et al*. 2013). The O-polysaccharide (OPS) of the Brucella smooth lipopolysaccharide is almost identical with that of bacteria causing false positive serological reactions particularly Y. enterocolitica O:9 (Szulowski *et al*. 2013; Weiner *et al*. 2013). The serological tests (RBPT and i-ELISA) used in this study detect antibodies to this antigen and their specificity would be compromised as they cannot distinguish between antibodies raised against brucella and co-infecting bacteria carrying the cross-reacting OPS. Szulowski *et al*., (2013) had emphasized the need for establishing clear techniques and guidelines to deal with such cases. Typical cross-reactions and detection of serotypes of Yersinia enterocolitica have been confirmed in pig herds with a high degree of false positive *Porcine Brucellosis* reactions (Szulowski *et al*. 2013; Weiner *et al*. 2013). False positive serological reactions have been reported to occur with moderate to high prevalence throughout Europe and other parts of the world (Pouillot *et al*. 1998; Szulowski *et al*. 2013) and could also be present in Cameroon. It is possible that the apparent prevalence observed in this study was influenced by false positive serology reactions of cross reacting bacteria. Though herd size, season, presence of other species (mix-husbandry) and previous false positive serological reactions in a given herd were significant risk factors at herd level screening for *Bovine Brucellosis*, herd size, sex and breed did not seem to be associated with the appearance of false positive reactions at the individual animal level and young animals were significantly more seropositive for *Brucellosis* than older ones (Pouillot *et al*. 1998).

The implementation of bacteriological examination for the presence of cross-reacting bacteria and isolation of these bacteria would be essential data for proper interpretation of *Porcine Brucellosis* serological test results. Nonetheless, RBPT is internationally recommended for screening of large numbers of sera while ELISA offers the highest

sensitivity and specificity of all currently available serological tests (Corbel 2006; Algers *et al*. 2009; Nielsen & Yu 2010; Muñoz *et al*. 2012; Praud *et al*. 2012). This study used RBPT and i-ELISA in combination to minimize the possible measurement of false positive errors and revealed that *Porcine Brucellosis* is a real pig and human health problem in piggery structures in Cameroon. Since indigenous pigs were *Brucellosis* seronegative, a bacteriological study of *Porcine Brucellosis* would be essential to investigate the nature of *Brucellosis* in indigenous pigs as well as determine the serotypes circulating in improved pig breeds in the country. Public awareness campaigns and health education especially among livestock professionals and in agropastoral communities should be highlighted to disseminate knowledge, potential risk factors and protective measures against zoonotic *Brucellosis*. The sensitization of animal professionals to improve their level of awareness as well as the need for intensification of the integrated "One Health" approach for effective management in the country should be emphasized.

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EFFECTS OF BROODING HEAT SOURCES ON GROWTH PERFORMANCE AND COST OF FEED UTILIZATION OF TWO STRAINS OF BROILER CHICKENS

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Abstract

This study investigated the effects of three brooding heat sources (charcoal pot, kerosene stove and lantern) on the growth performance and cost of feed utilization of two strains (Marshal MY and Hubbard) of broiler chickens. A total of 198 broiler chickens (99 birds each of Marshal MY and Hubbard) were used for the study for a period of 3 weeks. The experiment was arranged in a 2x3 factorial layout, consisting of 6 treatment groups with 3 replications each and 11 birds per replicate. The birds were managed intensively on deep litter with the provision of feed and water *ad libitum*. Data were obtained on the growth performance and cost efficiency of feed utilization on a weekly basis. The data recorded were subjected to analysis of variance in a completely randomized design. The results showed significant ($P<0.05$) differences in feed intake, feed conversion ratio, protein efficiency, cost of feed utilization and total cost of production with respect to the brooding heat sources in the main effects. The best feed conversion ratio of 1.58 was obtained in birds brooded using a charcoal pot as a source of heat. However, no significant ($P>0.05$) differences were obtained in all the parameters considered in the main effects of the strains. In the interactive effects of heat sources and strains, significant ($P<0.05$) differences were observed in the feed intake, feed conversion ratio, protein intake, protein efficiency ratio, cost of feed per kg and total cost of production per bird. Birds on a charcoal pot heat source recorded the best feed conversion ratio, highest protein efficiency and lowest cost of production per bird on the two strains of broiler chickens. It was concluded that charcoal pots can be used as a brooding heat source by small scale poultry farmers for better growth performance and economic viability of production.

Keywords: Brooding, Heat sources, Marshal MY, Hubbard and growth performance

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Introduction

Broiler chickens production is an important sub-sector of poultry production in Nigeria with enormous potential to bring about desired economic change. It has continually contributed positively in the areas of food provision (poultry meat), employment *generat*ion and a source of income to producers. In recent years there has been an increase in the demand for poultry products, this is due in part to the growing population and consumers' awareness of the nutritional advantages of poultry meat relative to other meats. In an attempt to increase broiler chicken production in Nigeria to meet demand, the roles of small scale poultry farmers are worthy of note. In Nigeria, small scale producers represent approximately 94 percent of total poultry keeping, and account for nearly four percent of the total estimated value of the livestock resources in the country (FAO, 2004). In this system, locally available resources are used to enhance production; essential management activities like brooding are practiced. Brooding, which encompasses the care and provision of supplemental heat to chicks is important to give the chicks a good start at the early developmental stage. Deaton *et al.* (1974) reported that the normally recommended brooding temperatures of 35^0C, 32^0C, and 29^0C for the 1st, 2nd, and 3rd weeks, respectively, could be reduced to 29^0C, 27^0C, and 24^0C when warm-room brooding is used. Carr *et al.* (1974) stated that a brooding temperature as low as 27^0C during the first week was adequate in warm-room brooding systems. Fawwad *et al.* (2008) compared the effects of three brooding techniques (gas, electric and wood) on broiler chicken performance. They recorded significant differences in weight gain, feed consumption, feed conversion ratio, respiratory rate and recommended gas brooding as an economical technique to enhance the productive performance of birds. Alternative heat sources such as charcoal pots, kerosene lanterns and stoves can be adapted by small scale poultry producers in Nigeria because of the high cost and unavailability of sophisticated brooding equipment. Therefore, this study investigated the effects of three heat sources on growth performance and cost efficiency of feed utilization of two strains of broiler chickens.

Materials and Methods

Experimental location

The experiment was carried out at the poultry unit of the Directorate of University Farms (DUFARMS) and the Animal Products and Processing Laboratory of the Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site falls within latitude 7°13' N and longitude 3°25' E (Google earth, 2015).

Experimental Birds and Management

A total of 198 day old broiler chickens (99 birds each of Marshal MY and Hubbard) were purchased from a reputable hatchery and used for the experiment for a period of three weeks. The birds were brooded for three weeks using three different brooding heat sources: charcoal pot, kerosene lantern and kerosene stove. Birds were managed intensively (Deep-litter) with the provision of commercial feed (starter diet) and water ad-libitum. All the routine management practices relevant to broiler chickens were dully observed.

Description of the brooding heat sources

Charcoal pot: Small size charcoal pots with a diameter of 25cm were loaded with charcoal and lit, the charcoal was allowed to burn completely to *generat*e heat for brooding before placement inside the individual treatment replications during brooding. The charcoal pots were guarded on the sides to prevent chicks from having direct contact. The behaviour of the chicks was regularly monitored as an indicator for adjusting the heat appropriately. Efforts were made to regulate the temperature between 32 - 37°C. However, the regulated temperature remained at 36^0C

Kerosene lantern: Big size kerosene lanterns were used for the experiment, the lanterns were always lit and refilled with kerosene all through the experimental period.

The heat *generat*ed from the lanterns radiated within the brooding unit and the behavioural response of the chicks was used as an indicator for regulating the kerosene lanterns. Efforts were made to regulate the temperature between 32 - 37°C. However, the regulated temperature remained at about 30°C.

Kerosene stove: Medium size kerosene stoves were used for the study with a flat metal plate placed on top of the stoves to allow for uniform radiation of heat *generat*ed within the brooding unit. The responses of the chicks were also used for regulating the heat *generat*ed within the brooding compartment. Efforts were made to regulate the temperature between 32 - 37°C. However, the regulated temperature remained at 33°C.

Experimental Design

The experiment consisted of a total number of 198 broiler chickens (99 birds each of Marshal MY and Hubbard) laid out in a 2x3 (two strains of broiler chickens and three brooding heat sources) factorial experimental arrangement of 6 treatment groups with 3 replications each in a Completely Randomized Design.

Data Collection

Data were collected on growth performance and cost of feed utilization parameters to achieve the objectives of the study.

Growth Performance parameters

1. *Feed intake:* this was recorded weekly for each replicate. Feed left over was subtracted from the amount of feed offered to the birds weekly to determine the feed intake.

Feed intake (g) = total feed offered (g) – left over feed (g)

Average feed intake (g/bird) = feed intake/ number of birds

2. *Body weight gain:* was also recorded for each replicate. The average weight gain per bird was noted by deducing the difference between the final body weight and initial body weight and dividing this value by the number of birds per replicate.

Average body weight gain (g/bird) = (final weight (g) – initial weight (g))/number of birds

3. *Feed conversion ratio (FCR):* was calculated by finding the ratio of the feed intake to the body weight gain.

FCR = total feed consumed (g) / body weight gain (g)

4. *Mortality (%):* was calculated as the ratio of the number of dead birds to the total number of birds per replicate, expressed as a percentage.

*% Mortality = (number of dead birds/total number of birds alive)*100*

5. Protein efficiency ratio (PER) = weight gain (g)/ protein fed (g)

Cost of feed utilization parameters

The prevailing market prices of materials used in the experiment (□170.93 = $1) were used in the calculation of the cost.

1. Cost of feed per kg (N) = Quantity (kg)*Cost of unit ingredient (N)
2. Total cost of production per bird (N) = Cost of feed intake, water intake, medication and vaccination, and labour

Statistical Analysis

Data obtained were subjected to analysis of variance in a Completely Randomized Design. Significantly ($P<0.05$) different means were separated using Duncan's Multiple Range Test (SAS, 2000).

Results

The main effects of brooding heat sources on the performance of two strains of broiler chickens is shown in Table 1. The results showed no significant ($P>0.05$)

differences in all the growth performance and cost benefit parameters with respect to the two strains of broiler chickens considered. However, significant (P<0.05) differences were obtained in the effects of the brooding heat sources, on feed intake, feed conversion ratio, protein intake, protein efficiency ratio, cost of feed consumed per kg and the total cost of production per bird. Kerosene stoves had the highest feed intake value of 23.76 g/bird/day though not significantly (P>0.05) different from kerosene lanterns with 22.81 g/bird/day and the lowest (20.90 g/bird/day) feed intake was obtained in birds with charcoal pots as a source of heat . Significant (P<0.05) differences were observed in the feed conversion ratio (FCR) with respect to the brooding heat sources. Birds on charcoal pot heat sources recorded the best feed conversion ratio (1.58) relative to the ones on kerosene lanterns (1.78) and stoves (1.82), respectively. A similar trend was observed in the protein efficiency ratio (PER) with significant (P<0.05) differences across the brooding heat sources. The highest protein efficiency ratio (2.85) was recorded from birds on charcoal pots as against the PER of the birds on kerosene lanterns (2.53) and kerosene stoves (2.37) which were statistically similar.

There was a significant (P<0.05) difference in cost per kg of feed consumed between the brooding heat sources. Kerosene stoves recorded the highest cost (N41.92/kg), which was not significantly (P>0.05) different from that of kerosene lanterns with (N36.87/kg). The lowest cost of feed consumed per kg (N36.87/kg) was obtained in birds with charcoal pots as the source of heat. The results also showed a significant (P<0.05) difference in the total cost of production per bird with respect to the brooding heat sources. Birds under kerosene stoves recorded the highest cost of production (N432.58) per bird, followed by kerosene lanterns (N347.96) per bird. The lowest cost of production (N216.00) per bird was obtained in birds with charcoal pots as a brooding heat source.

Table 2 shows the interactive effects of brooding heat sources on the performance of two strains of broiler chickens. There was a significant (P<0.05) difference in the feed intake with respect to brooding heat sources and strains of broiler chickens. The feed conversion (FCR) ratio showed a significant (P<0.05) different across the brooding heat sources in relation to the strains of broiler chickens. The highest (1.88) FCR was obtained on the Marshal strain brooded with kerosene lanterns while the best FCR was obtained on the two

Table 1: Main effects of brooding heat sources on performance of two strains of broiler chickens

Parameter	Marshal MY	Hubbard	SEM	Charcoal pot	Kerosene lantern	Kerosene stove	SEM
Initial weight (g/bird)	14.37	18.63	0.00	16.50	16.50	16.50	0.00
Final weight (g/bird)	286.03	296.07	2.01	296.99	286.95	291.21	3.44
Weight gain (g/bird/day)	12.93	13.20	0.09	13.34	12.78	13.08	0.16
Feed intake (g/bird/day)	22.78	22.21	0.23	20.90^{b}	22.81^{a}	23.76^{a}	0.29
Feed conversion ratio	1.77	1.68	0.01	1.58^{b}	1.78^{a}	1.82^{a}	0.02
Protein intake (g/bird/day)	5.11	4.95	0.06	4.69^{b}	5.08^{a}	5.33^{a}	0.07
Protein efficiency ratio	2.55	2.62	0.02	2.85^{a}	2.53^{b}	2.37^{b}	0.05
Cost of feed consumed (N/kg)	40.17	38.98	0.41	36.87^{b}	39.95^{a}	41.92^{a}	0.52
Total cost of production (N/bird)	332.72	331.64	0.48	216.00^{c}	347.96^{b}	432.58^{a}	0.56
Mortality (%)	4.00	4.63	0.93	3.00	1.50	1.50	1.23

^{abc} *Means in the same row with different superscript differ significantly (P<0.05)*

Table 2: Interactive effects of brooding heat sources on performance of two strains of broiler chickens

Heat Source / Strain	Charcoal Pot		Kerosene lantern		Kerosene stove		SEM
	Marshal MY	Hubbard	Marshal MY	Hubbard	Marshal MY	Hubbard	
Initial weight (g/bird)	14.37	18.63	14.37	18.63	14.37	18.63	0.00
Final weight (g/bird)	290.61	303.36	275.95	293.94	291.52	290.91	8.42
Weight gain (g/bird/day)	13.15	13.54	12.45	13.11	13.19	12.96	0.40
Feed intake (g/bird/day)	20.81^{b}	20.99^{b}	23.43^{a}	22.19^{ab}	24.08^{a}	23.44^{a}	0.70
Feed conversion ratio	1.60^{c}	1.55^{c}	1.88^{a}	1.67^{bc}	1.83^{ab}	1.81^{b}	0.06
Protein intake (g/bird/day)	4.66^{b}	4.71^{b}	5.25^{a}	4.90^{ab}	4.40^{a}	5.25^{a}	0.16
Protein efficiency ratio	2.83^{ab}	2.88^{a}	2.38	2.68^{abc}	2.44^{bc}	2.30^{c}	0.13
Cost of feed consumed (N/kg)	36.70^{b}	37.03^{b}	41.33^{a}	38.56^{ab}	42.48^{a}	41.35^{a}	1.26
Total cost of production (N/bird)	216.33^{c}	215.66^{c}	345.19^{b}	347.24^{b}	433.15^{a}	432.02^{a}	1.37
Mortality (%)	6.00	3.42	3.00	4.63	3.00	2.50	3.00

^{abc} *Means in the same row with different superscript differ significantly (P<0.05)*

strains of chicks (1.60, 1.55 for Marshal and Hubbard, respectively) brooded with charcoal pots. The result on the protein intake showed a significant (P<0.05) difference between the strains under the charcoal pots and strains under both kerosene lanterns and kerosene stoves.

Significant (P<0.05) difference was observed in the cost per kg of feed with respect to brooding heat sources and strains of broiler chickens. Cost of feed consumed per kg was highest for Marshal and Hubbard raised under kerosene lantern and kerosene stove respectively, while the lowest cost of feed consumed per kg (36.70 and N37.03/kg for Marshal and Hubbard, respectively) was obtained on birds brooded under charcoal pot. This supports the findings of May *et al.* (1997). .

In the interaction between brooding heat sources and strains, the total cost of production per bird was significantly (P<0.05) affected. Birds brooded on charcoal pots recorded the lowest cost on the two strains (N216.33, N215.66/bird for Marshal and Hubbard, respectively) relative to kerosene lanterns and kerosene stoves. The highest cost of production per bird was recorded on the strains raised on kerosene stoves (N 433.15, N432.02/bird for Marshal and Hubbard, respectively).

Discussion

The variation in the feed intake with the brooding heat sources could be due to the inconsistency in the brooding temperature *generat*ed by the heat sources particularly on birds brooded with charcoal pots. This is in line with the findings of Harris *et al.* (1975); Orban and Roland (1990) who reported that

fluctuations in brooding temperature results in less feed consumption. Consumption of more feed in the birds brooded with Kerosene lanterns and stoves may also be due to higher fluctuations in temperature which might have resulted in cold temperatures inside the shed and birds consuming more feed.

The results on FCR and PER suggest that birds on the different heat sources were exposed to varying brooding temperatures which may be low as the case of kerosene lanterns and stoves with higher feed consumption and poorer FCR, this supports the findings of Renwick and Washburn (1982); Buys *et al*. (1999) who stated that low brooding temperature results to more feed intake and poor feed conversion. Conversely, birds on charcoal pots recorded a considerable lower feed intake and the best FCR which must have resulted from a uniform brooding temperature, leading to improved feed conversion ratio.

The variation on the cost per kg of feed consumed can be attributed to varying brooding temperatures achieved during the experimental period. This supports the findings of Brian (2005) who reported that chicks exposed to low heat consumed more feed and increased feed cost. This implies that a better brooding temperature was attained in the birds on charcoal pots brooding heat source. Also, the results on the cost of production per bird were due to variations in the additional cost of fueling the brooding heat sources. This is in line with the findings of May *et al*. (1997) who reported that broilers exposed to low heat had increase feed intake due to increase metabolic heat production and maintain internal regulation resulting in slower growth, more feed conversion and increased cost of feeding and overall cost of production.

In the interaction effects, birds brooded with kerosene stoves and kerosene lanterns had a higher feed intake in both Marshal and Hubbard strains relative to a lower feed intake recorded in chicks brooded under charcoal pots (20.81 and 20.99 g/bird/day for Marshal and Hubbard respectively). This is due to the attainment of a better brooding temperature on the two strains of broiler chicks brooded with charcoal pots. The results on the FCR suggest that the brooding temperature provided by the charcoal pots on the two strains of broiler chickens was sufficient to enhance a better feed conversion. The result on the protein intake follows the same trend as that obtained on the feed intake as indicated in the findings of Brian (2005).

Conclusion and Recommendation

Conclusion

The study showed no significant differences in the final weights of chicks at the end of the brooding period which implies that, any of the three brooding heat sources could be used in brooding broiler chickens. However, the use of charcoal pots as a heat source in brooding Hubbard strain of broiler chickens was best. In addition, the significance shown in the total cost of production of birds implies that charcoal pots were the most cost effective means of brooding.

Recommendation

Charcoal pots are recommended in brooding broiler chicks for economic benefits in small scale broiler chickens production.

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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
March 2018

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- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

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