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EFFECT OF DIETARY CALCIUM LEVEL ON EGG PRODUCTION AND EGG SHELL QUALITY IN BROILER BREEDER HENS FROM 36 TO 60 WEEKS OF AGE

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ABSTRACT: *This study was conducted to evaluate the effects of different calcium (Ca) levels in diet on shell quality and egg production of Ross broiler breeder hens from 36 to 60 weeks of age. A total of 198 pullets were reared on restricted diets with 1.0, 1.5 and 2.0% Ca, up to 22 weeks. The pullets in each experimental diet were further randomly divided into three treatments with 1.5, 2.5 and 3.5% dietary Ca (66 birds per treatment) and fed from 23 to 60 weeks. The hens were caged individually. The cages were fitted with feed troughs and water nipples. Birds were fed breeder diet (23 to 34 weeks), breeder diet phase 2 (35 to 46 weeks) and breeder diet phase 3 (47 to 60 weeks) feed intake was administered according to Ross Breeders recommendations. The rations were isocaloric and isonitrogenous with different levels of Ca and P and were administered according to Ross Breeders Recommendations. Dietary treatment significantly (P<0.0001) affected Ca intake of broiler breeder hens. An average Ca intake (g/hen/day) of 2.14, 3.76 and 5.39 for the 1.5, 2.5 and 3.5% Ca levels, respectively occurred during the experimental period. Egg production, egg weight, egg mass, egg contents and shell weight/unit surface area (SWUSA), shell weight, shell percentage and shell thickness increased (P<0.0001) when dietary Ca was raised from 1.5% to 2.5%. However, no significant (P>0.05) differences were found in these variables between 2.5 and 3.5% Ca levels. All the eggshell quality variables increased over time while egg mass and egg production declined. From the results of the present study, it is concluded that increasing Ca level from 1.5 to 2.5% could improve eggshell quality. Present results suggest that the 2.5% Ca (3.8 g Ca/hen/day) seems to be adequate to support egg production and improving eggshell quality of broiler breeders.*

ORIGINAL ARTICLE

Keywords: *Calcium, egg production, eggshell quality, egg weight, phosphorus,*

INTRODUCTION

The ability of hens to produce quality shells depends largely on the availability of calcium (Ca) from ingested food and skeleton (Farmer et al., 1983). It is claimed (Klasing, 1998) that the amount of dietary Ca required for maximising bone or eggshell mineralisation and strength is greater than that needed for other functions. Therefore, proper build-up of Ca stores is essential for the maintenance of bone integrity and acceptable shell quality (Robinson, 1999).

The term "shell quality" is frequently used as a synonym for "shell strength", and denotes the ability of eggshells to withstand externally applied forces without cracking or breaking (Hamilton, 1982). Eggshell quality can be defined by variables such as egg specific gravity (ESG) through its relationship to shell porosity as shown by positive correlation with pore concentration (Peebles and Brake, 1987). Factors that affect the strength of eggshells are heredity, clutch position, rate of production (Hammerle, 1969) age, health status (disease), season, temperature, nutrition (Hammerle, 1969; Wolford and Tanaka, 1970), strain of hen, time of the day eggs are laid, eggshell

ultrastructure (Hamilton et al., 1979a,b), housing system, length of lay and neuro-humoral reproductive control mechanisms (Wolford and Tanaka, 1970). The most common physical properties associated with eggshell strength are shell thickness and shell egg specific gravities. Richards and Staley (1967) suggested that shell thickness, shell weight, shell percentage and shell weight per unit surface area (SWUSA), may be classified as shell quality measurements, as these variables are significantly ($P<0.01$) correlated with each other.

As the hen gets older, minimisation of handling loss by maintenance of eggshell quality becomes more important than egg weight. Shell quality is governed by the quantity of the SWUSA of the egg. Shell deposition increases for several months and then plateaus, as the hen gets older. Since egg weight increases throughout the laying period, once shell deposition plateaus, shell quality declines. In this regard, dietary Ca content is important as shell deposition and quality are directly related to the level of Ca in the diet (Ousterhout, 1980).

Because feed intake is restricted in broiler breeder hens, with most feed being consumed during the early morning hours, these and especially older breeder hens may be more susceptible to periods of Ca deficiency during shell formation than hens that are fed *ad libitum* (Farmer et al., 1983). The present study was undertaken to gain additional information on the effects of three dietary Ca levels on shell quality and egg production of broiler breeder hens from 36 to 60 weeks of age.

MATERIALS AND METHODS

Birds and husbandry

A total of 198 Ross broiler breeder pullets were reared on restricted diets with 1.0, 1.5 and 2.0% Ca treatment up to 22 weeks of age. The pullets in each experimental diet were further randomly divided into three treatments with 1.5, 2.5 and 3.5% dietary Ca (66 birds per treatment) and fed from 23 to 60 weeks. The hens were placed in individual cages within a common room. The cages were fitted with feed troughs, water nipples and perches. The hens were photo stimulated at 22 weeks of age and received 16 hours of light starting from week 26 of age. This photo schedule was continued to 60 weeks of age.

Birds were fed pre-breeder diet from 19 to 22 weeks of age, breeder diet phase 1 (23 to 34 weeks), breeder diet phase 2 (35 to 46 weeks) and breeder diet phase 3 (47 to 60 weeks). The feed intake was administered in accordance with Ross Breeders recommendations. Individual body weight measurements were taken on three weekly intervals for the duration of the experiment.

Experimental parameters measured

Egg parameters: Egg numbers were recorded daily and summarised on a weekly basis throughout the experimental period. Abnormal eggs having multiple yolks, shell-less and those with defective shells were recorded. Shell-less eggs were, however, not included in the weight data. Cumulative egg production was calculated on a per bird basis throughout the experimental period. Percent lay on daily basis was calculated using a formula given by North and Bell (1990).

Individual egg weights were recorded for all the eggs produced by each hen on daily basis throughout the test period. After the mean egg weight had been determined in grams each, daily egg mass was computed by multiplying percent hen day production by mean egg weight (North and Bell, 1990).

Eggshell quality: Eggshell thickness was determined according to the procedures of Ehtesham and Chowdhury (2002) and Kul and Seker (2004). The eggshells from individual eggs were then placed in crucibles and dried overnight in the oven at 60 ° C and cooled in the desiccators for approximately 30 minutes, and weight was recorded. The weight of egg contents was calculated by subtracting shell weight from egg weight. Shell percentage was calculated by dividing dry shell weight by egg weight and multiplying by 100 (Chowdhury and Smith, 2001). The surface area (cm^2) of each egg was calculated using the formula of Carter (1975), $3.9782W^{.7056}$, where W is the egg weight in grams. Shell weight was divided by egg surface area to give the SWUSA expressed as mg/cm^2 (Wells, 1967).

Statistical analyses

Calcium level during the rearing period (one day old to 18 weeks) had no significant effect on bone measurements. Therefore, the effects of dietary Ca level and age on the egg characteristics data during laying period (36 to 60 weeks) were analysed as a 3 × 9 factorial block design in which data from individual birds (22 birds per treatment) served as replicates. Data were subjected to ANOVA using the General Linear Models procedure (SAS Institute, 1996) to assess the effect of dietary Ca level and age on response variables relating to egg production, egg mass, egg weight, shell thickness, shell weight and shell percentage. The differences between treatment means were separated using Tukey's studentised range (Honestly Significant Difference, HSD) test.

RESULTS AND DISCUSSION

Calcium intake

As illustrated in Table 1, dietary Ca levels had a significant ($P<0.0001$) effect on Ca intake of the hens throughout the laying period. An average Ca intake (g/hen/day) of 2.14, 3.76 and 5.39 for the 1.5, 2.5 and 3.5% Ca levels, respectively occurred during the experimental period (36 to 60 weeks). Ca intake in the hens increased

(P<0.05) as dietary Ca was concentration raised from 1.5 to 3.5%. These results are consistent with those of Clunies (1992a) and Keshavarz and Nakajima (1993) who fed laying hens Ca levels ranging from 2.5 to 5.5% and found that Ca intake increased with increasing dietary level of Ca. A significant (P<0.0001) Ca level × age interaction occurred.

Low Ca intake values of hens were noted at 39 weeks compared to other age periods (Table 1). High ambient temperature above the comfort zone could be a contributory factor to the lower Ca intake values during this period. The average maximum temperatures at 36, 39 and 42 weeks were 32.6, 35.6 and 30.9 ° C, respectively. The Ca intake of hens did not appear to decline with age until 54 weeks where after Ca intake started to decline.

Table 1 - Effect of dietary calcium levels and age on calcium intake of broiler breeder hens

Age (weeks)	Dietary level of calcium		
	1.5%	2.5%	3.5%
36	2.27±0.06[a]	3.90±0.06[b]	5.50±0.06[c]
39	1.91±0.06[a]	3.42±0.06[b]	4.77±0.06[c]
42	2.18±0.06[a]	3.81±0.06[b]	5.82±0.06[c]
45	2.28±0.06[a]	3.99±0.06[b]	5.78±0.06[c]
48	2.12±0.06[a]	3.59±0.06[b]	5.21±0.06[c]
51	2.19±0.06[a]	3.92±0.06[b]	5.62±0.06[c]
54	2.18±0.06[a]	4.02±0.06[b]	5.88±0.06[c]
57	2.09±0.06[a]	3.65±0.06[b]	5.18±0.06[c]
60	2.03±0.06[a]	3.51±0.06[b]	5.18±0.06[c]
CV%	10.9		
Significance level (P)			
Treatment	0.0001		
Age	0.0001		
Interaction	0.0001		

[a,b,c]Means within a rows with no common superscripts differ significantly (P<.05).

Egg production

Dietary Ca level had a significant (P<0.001) effect on egg production. From the results of current study, it is evident that hen day egg production from week 36 for birds fed 1.5% Ca diets was in general significantly (P<0.05) lower than those fed the 2.5 and 3.5% Ca diets. These results are consistent with reports of Ahmad et al. (2003) and Clunies et al. (1992b) in commercial laying hens. Accordingly, Atteh and Leeson (1983) and Manley et al. (1980) reported no effect of feeding Ca levels ranging from 2.5 to 4.2% on egg production in commercial laying hens and turkey breeder hens. The mean hen day percent production values during the entire laying period for the 1.5, 2.5 and 3.5% dietary Ca levels were 62.84 ± 0.49, 66 ± 0.47 and 67.46 ± 0.46, respectively. The hens received 3.5% dietary Ca laid at a rate 1.7% higher than those that received 2.5% Ca diets. The average number of eggs produced by each hen from 25 to 60 weeks of age (245 days) for the 1.5, 2.5 and 3.5 Ca levels was 150.21 ± 3.24, 161 ± 3.14 and 165 ± 3.11, respectively. The yearly egg production from birds fed 2.5 and 3.5% Ca diets was in agreement with Ciacciariello and Gous (2002) who reported that a broiler breeder hen produces about 165 eggs in the 60 weeks of production life.

Rose (1997) reported the decline in egg production immediately after peak production to lengthening of egg formation time. A slow and continuous reduction in the rate of egg yolk deposition as the bird age also contributes to a decline in egg production.

The effect of Ca level and age on egg mass is shown in Figure 1. In accordance with egg production egg mass was significantly (P<0.05) lower for birds fed 1.5% Ca diets compared to 2.5 and 3.5% Ca diets (Figure 1). No statistical (P>0.05) differences were observed between the 2.5 and 3.5% dietary Ca levels. As shown in Figure 1, egg mass in accordance with egg production significantly (P<0.0001) declined with age. This is in agreement with Rose (1997) who stated that egg mass output rises to a peak shortly after a flock has reached peak egg production and thereafter decline steadily until egg production ceases.

Mortality

Ali et al. (2003) reported that mortality plays a major role in determining performance of the broiler breeder enterprise, as it is a function of the dead and culled birds over the growth and production period. Higher mortality has been associated to adversely affect laying performance of broiler breeders. In the present study, mortality included birds that died or culled from a flock during the second laying cycle (i.e., 36 to 60 weeks). Nine hens (3 from each treatment) died during this phase, indicating that Ca level had no effect on mortality. Atkinson (1967), Pepper et al. (1968) and Scott et al. (1999, 2000) observed similar results when they fed turkey hens and Shaver Starcross hens diets supplemented with different levels of Ca (arranged from 1.24 to 6.0%).

Eggshell quality

The mean values for egg weight (g), shell weight (g), egg contents (g), egg surface area (cm^2), shell percentage shell, SWUSA (mg/cm^2) and eggshell thickness (sharp end, equator and broad end) (mm) are presented in Table 2. As illustrated in Table 2, the feeding of 2.5 and 3.5% Ca diet resulted in greater egg weight, shell weight,

egg contents, egg surface area, shell percentage and shell thickness than the lower level (1.5% Ca). No significant (P>0.05) differences in these variables were found between the 2.5 and 3.5% Ca diet levels.

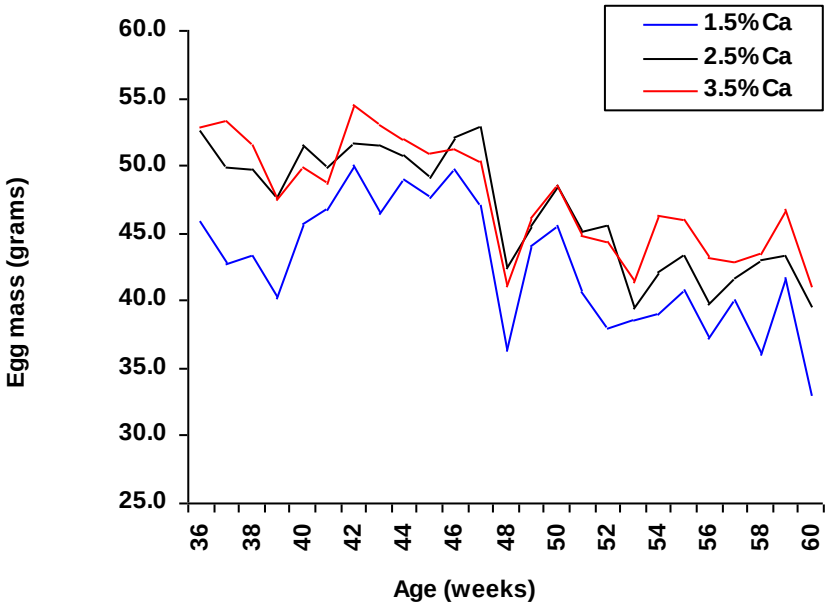

Fig. 1- Effect of dietary Ca levels and age on egg mass

Egg weight tended to increase by about 1.0% as Ca level increased from the 2.5 to 3.5%. The results of the present study agree with the findings of Summers et al. (1976) who reported linear increase in egg weight with higher level of Ca (2.96 *vs*. 1.50%). These results are also in accordance with Ahmad *et al*. (2003), Atteh and Leeson (1983), and Zapata and Gernat (1995) who reported that increasing dietary Ca level from 2.5 to 5.0, 3.0 to 4.2 and 3.0 to 3.5%, respectively had no effect on egg weight. These results are, however, in partial agreement with previous reports of Reddy et al. (1968), Roland et al. (1996) and Scott et al. (2000) who reported either non-significant differences in egg weight or significant (P>0.05) decreases in egg weight due to feeding increased levels of Ca. These results are, however, in contrast with the results of the first phase of the laying cycle (25 to 35 weeks), where no significant (P<0.05) differences in egg weight was observed due to increases in dietary Ca level. According to these results, the effect of different dietary Ca levels on egg weight appears during the later stages of the laying period.

As already mentioned, egg contents significantly (P<0.0001) increased as dietary Ca concentration increased from 1.5 to 2.5%. Thereafter, further increases resulted in non-significant (P>0.05) increase in egg contents (Table 2). An increase in egg contents could be attributable to increases in egg and shell weights since egg contents are a function of these two parameters.

As already mentioned, egg contents significantly (P<0.0001) increased as dietary Ca concentration increased from 1.5 to 2.5%. Thereafter, further increases resulted in non-significant (P>0.05) increase in egg contents (Table 2). An increase in egg contents could be attributable to increases in egg and shell weights since egg contents are a function of these two parameters.

As indicated in Table 2 egg surface area was significantly (P<0.05) influenced by dietary Ca level up to 2.5%. Thereafter, there were no further statistically significant (P>0.05) increases in egg surface area. Egg surface area significantly (P<0.0001) increased over time (Table 2). The increase in egg surface area could be attributable to increases in egg weight due to age. These two variables are highly positively correlated (r = 1.00).

It was observed that shell percentage tended (P>0.05) to plateau as dietary Ca level increased from 2.5 to 3.5% and this occurred between 36 and 39 weeks of age. Thereafter, shell percentage was not significantly (P>0.05) different among Ca levels (Table 2). These results are in disagreement with those of Reddy et al. (1968) who reported that feeding commercial layers diets containing 3.85 and 3.05% Ca resulted in significantly (P<0.05) greater shell percentage than lower levels (i.e., 2.25% and 2.65% Ca). From 25 to 35 weeks, shell percentage increased significantly (P<0.05) with increased Ca level. The study of Kul and Seker (2004) in quails demonstrated that shell percentage decreased with increased egg weight. Similar observations were made in the current study.

From Table 2 it is evident that the SWUSA was significantly (P>0.05) lower for 1.5% Ca diets compared to 2.5 and 3.5% Ca diets. The SWUSA was not significantly (P>0.05) different among dietary Ca levels at 42, 45, 48, 51, 54 and 60 weeks of age. These results are consistent with those of Ousterhout (1980) who reported that SWUSA increased by 1.25 mg when dietary Ca was increased by 1.0% (*i.e*., from 3.75 to 4.75%). In the present study, SWUSA increased by 1.62 mg/cm^2 as Ca level increased from 1.5 to 2.5%. Table 2, illustrated that dietary Ca level had a significant (P<.05) effect on SWUSA only at 36 and 39 weeks of age. These results agree with findings of Nordstrom and Ousterhout (1982) who reported that SWUSA decreased significantly (P<.05) with increasing egg weight.

Table 2 - The effect of dietary calcium level and age on egg weight and eggshell parameters

		Age in weeks										Significance of effect (P)			
Variable	**Treatment**	**36**	**39**	**42**	**45**	**48**	**51**	**54**	**57**	**60**	**Means**	**Treatment**	**Age**	**Interaction**	**CV**
Egg weight (g)	1.5% Ca	62.75±0.66	62.86±0.77	66.95±0.65	69.72±0.69	71.09±0.79	71.59±0.82	72.25±0.87	72.62±0.87	73.41±1.16	69.25±0.27^{a}	0.0001	0.0001	0.9830	6.2
	2.5% Ca	64.15±0.61	65.21±0.65	68.12±0.63	70.86±0.69	71.74±0.69	72.70±0.75	74.55±0.78	74.09±0.87	73.73±0.95	70.57±0.25^{b}				
	3.5% Ca	64.99±0.62	65.70±0.69	68.89±0.65	71.14±0.68	72.24±0.66	73.85±0.72	73.76±0.73	73.74±0.79	75.96±0.92	71.14±0.24^{b}				
	Means	63.96±0.36^{a}	64.59±0.41^{a}	68.00±0.37^{b}	70.57±0.40^{c}	71.69±0.41^{c}	72.71±0.44^{cd}	73.52±0.46^{cd}	73.48±0.49^{cd}	74.36±0.59^{d}					
Shell weight (g)	1.5% Ca	5.12±0.07^{a}	5.00±0.09^{a}	6.02±0.07^{a}	6.14±0.08^{a}	6.11±0.09^{a}	6.29±0.09^{a}	6.41±0.10^{a}	6.16±0.10^{a}	6.31±0.13^{a}		0.0001	0.0001	0.0001	8.0
	2.5% Ca	5.70±0.07^{b}	5.71±0.07^{b}	6.02±0.07^{a}	6.30±0.08^{a}	6.32±0.08^{ab}	6.49±0.09^{a}	6.63±0.09^{a}	6.54±0.10^{b}	6.46±0.11^{a}					
	3.5% Ca	5.81±0.07^{b}	5.79±0.08^{b}	6.07±0.07^{a}	6.26±0.07^{a}	6.40±0.07^{b}	6.43±0.08^{a}	6.56±0.08^{a}	6.54±0.09^{b}	6.69±0.10^{a}					
Egg contents (g)	1.5% Ca	57.63±0.62	57.87±0.72	60.93±0.61	63.58±0.65	64.98±0.75	65.30±0.77	65.84±0.82	66.45±0.82	67.10±1.09	63.30±0.26^{a}	0.0001	0.0001	0.9900	6.4
	2.5% Ca	58.45±0.57	59.51±0.62	62.10±0.60	64.56±0.65	65.42±0.65	66.21±0.71	67.92±0.73	67.55±0.82	67.27±0.89	64.33±0.23^{b}				
	3.5% Ca	59.18±0.58	59.91±0.65	62.82±0.62	64.88±0.64	65.83±0.62	67.43±0.68	67.20±0.69	67.19±0.75	69.26±0.87	64.86±0.23^{b}				
	Means	58.42±0.34^{a}	59.10±0.38^{a}	61.95±0.35^{b}	64.34±0.37c	65.41±0.39^{cd}	66.31±0.42^{de}	66.99±0.43^{de}	67.07±0.46^{de}	67.87±0.55^{e}					
Egg surface area (cm^{2})	1.5% Ca	73.78±0.53	73.87±0.62	77.24±0.52	79.48±0.56	80.58±0.64	80.98±0.66	81.51±0.70	81.79±0.70	82.42±0.93	79.07±0.22^{a}	0.0001	0.0001	0.9823	4.4
	2.5% Ca	74.93±0.49	75.79±0.53	78.17±0.51	80.37±0.56	81.09±0.55	81.85±0.61	83.32±0.63	82.94±0.70	82.66±0.76	80.12±0.20^{b}				
	3.5% Ca	75.62±0.50	76.20±0.56	78.79±0.53	80.60±0.55	81.48±0.53	82.76±0.58	82.69±0.59	82.67±0.64	84.40±0.74	80.58±0.19^{b}				
	Means	74.77±0.29^{a}	75.29±0.33^{a}	78.07±0.30^{b}	80.15±0.32^{c}	81.05±0.33^{cd}	81.86±0.36^{de}	82.51±0.37^{de}	82.47±0.39^{de}	83.16±0.47^{e}					
Shell percentage (%)	1.5% Ca	8.17±0.09^{a}	7.95±0.11^{a}	9.01±0.09^{a}	8.82±0.10^{a}	8.60±0.11^{a}	8.78±0.12^{a}	8.91±0.12^{a}	8.50±0.12^{a}	8.62±0.16^{a}		0.0001	0.0001	0.0001	7.0
	2.5% Ca	8.90±0.09^{a}	8.77±0.09^{b}	8.85±0.09^{a}	8.90±0.10^{a}	8.82±0.10^{a}	8.94±0.11^{a}	8.94±0.11^{a}	8.85±0.12^{a}	8.80±0.13^{a}					
	3.5% Ca	8.96±0.09^{a}	8.83±0.10^{a}	8.86±0.09^{a}	8.81±0.10^{a}	8.87±0.09^{a}	8.74±0.10^{a}	8.91±0.10^{a}	8.89±0.11^{a}	8.82±0.13^{a}					
SWUSA[1] (mg/cm^{2})	1.5% Ca	69.39±0.79^{a}	67.55±0.91^{a}	77.97±0.77^{a}	77.30±0.83^{a}	75.82±0.94^{a}	77.59±0.98^{a}	78.58±1.03^{a}	75.36±1.03^{a}	76.67±1.38^{a}		0.0001	0.0001	0.0001	6.7
	2.5% Ca	76.07±0.72^{b}	75.33±0.78^{b}	77.04±0.75^{a}	78.37±0.83^{a}	77.90±082^{a}	79.29±0.90^{a}	79.53±0.93^{a}	78.84±1.03^{ab}	78.29±1.13^{a}					
	3.5% Ca	76.87±0.74^{b}	76.01±0.83^{b}	77.02±0.78^{a}	77.65±0.81^{a}	78.52±0.79^{a}	77.63±0.86^{a}	79.34±0.87^{a}	79.19±0.94^{b}	79.25±1.10^{a}					
Shell thickness Sharp end (mm x 10^{-2}))	1.5% Ca	37.04±0.41^{a}	37.57±0.47^{a}	41.67±0.40^{a}	42.08±0.43^{a}	39.87±0.49^{a}	39.89±0.51^{a}	40.12±0.54^{a}	39.13±0.54^{a}	39.43±0.72^{a}		0.0001	0.0001	0.0001	6.6
	2.5% Ca	40.19±0.37^{b}	40.95±0.40^{b}	41.77±0.39^{a}	42.50±0.43^{a}	40.70±0.42^{a}	40.44±0.47^{a}	41.24±0.48^{a}	40.95±0.54^{a}	40.40±0.58^{a}					
	3.5% Ca	40.73±0.38^{b}	41.57±0.43^{b}	41.48±0.40^{a}	42.41±0.42^{a}	40.73±0.41^{a}	39.95±0.45^{a}	40.65±0.45^{a}	40.82±0.49^{a}	40.73±0.57^{a}					
Equator (mm x 10^{-2})	1.5% Ca	36.29±0.39^{a}	36.72±0.45^{a}	40.68±0.38^{a}	41.24±0.41^{a}	38.99±0.46^{a}	39.26±0.48^{a}	40.26±0.51^{a}	38.95±0.51^{a}	39.44±0.68^{a}		0.0001	0.0001	0.0001	6.3
	2.5% Ca	39.51±0.35^{b}	40.39±0.38^{b}	40.95±0.37^{a}	41.94±0.41^{a}	40.38±0.40^{b}	39.79±0.44^{a}	40.65±0.45^{a}	40.73±0.51^{b}	40.29±0.55^{a}					
	3.5% Ca	40.00±0.36^{b}	40.82±0.41^{b}	40.84±0.38^{a}	41.75±0.40^{a}	40.09±0.39^{ab}	39.40±0.42^{a}	40.71±0.43^{a}	40.53±0.46^{ab}	40.55±0.54^{a}					
Broad end (mm x 10^{-2})	1.5% Ca	36.03±0.39^{a}	36.50±0.46^{a}	40.77±0.39^{a}	41.54±0.41^{a}	39.10±0.47^{a}	39.29±0.49^{a}	40.53±0.52^{a}	38.75±0.52^{a}	39.24±9.69^{a}		0.0001	0.0001	0.0001	6.4
	2.5% Ca	39.27±0.36^{b}	40.35±0.39^{b}	41.10±0.38^{a}	42.16±0.41^{a}	40.38±0.41^{a}	40.25±0.45^{a}	40.78±0.46^{a}	41.51±0.52^{b}	40.93±0.56^{a}					
	3.5% Ca	39.82±0.3^{b}	40.77±0.41^{b}	40.94±0.39^{a}	41.92±0.40^{a}	39.99±0.39^{a}	39.47±0.43^{a}	40.97±0.44^{a}	40.63±0.47^{b}	40.38±0.55^{a}					

[1]SWUSA – shell weight per unit surface area. Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred. Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

Increasing Ca levels from 1.5 to either 2.5 or 3.5% resulted in a greater response of shell thickness. However, it was noted that increasing Ca beyond 2.5% resulted in shell thickness tending to plateau. A significant (P<0.0001) Ca level × age interaction for shell thickness occurred indicating that the influence of dietary Ca on shell thickness varied during different periods. Menge et al. (1977) observed similar results in turkeys. They reported significantly increased beta-backscatter (BBS) counts; indicating increased eggshell thickness and density, which are the principal elements of shell quality. In agreement with these results, Waldroup *et al.* (1974) and Zapata and Gernat (1995) reported no beneficial effects of Ca level on eggshell thickness after feeding caged turkey breeder hens and commercial layers diets containing Ca levels ranging from 2.5 to 3.5%. Nordstrom and Ouster out (1982) stated that in order for shell thickness to increase, shell weight must increase, egg surface area must increase, or a combination of these two changes must occur. In the present study, a combination of these two changes occurred.

In accordance with the results of Sparks (1998), shell thickness in this study tended to decline with flock age (Table 2). Roland (1980) contended that shell thickness decreases with hen age because total shell deposition after the first three months of lay remains fairly constant while eggs continue to increase in size. This causes the shell to be spread thinner, forcing shell quality to decline. Eggshells were significantly (P<0.05) thinner for birds fed 1.5% compared to 2.5 and 3.5% Ca diets at weeks 36 and 39. Thereafter, no statistically significant (P>0.0001) differences were observed with respect to the sharp and broad ends. In the case of the equator, eggshells from birds fed 2.5% Ca diets were significantly (P<0.05) different compared to eggshells from birds on 1.5% Ca diets at 48 and 57 weeks of age. No significant (P>0.05) differences in eggshell thickness were found between 1.5 and 3.5%, and 2.5 and 3.5% Ca diets with respect to the egg's equatorial region. These results confirmed previously reported findings (North and Bell, 1990) that eggshell thickness declines with age.

Regardless of the Ca level in the diet, the egg weight, shell weight and egg contents and egg surface area increased (P<0.05) over time from 36 to 60 weeks of age (Table 2). In the current study, egg weight and shell weight increased by averages of 1.9% and 2.2% per month, respectively. The greatest (P<0.0001) increase was noted from 39 to 45 weeks (Table 2). A tendency for egg weight to decline was observed at 54 and 57 weeks of age.

CONCLUSION

The results of the present study suggest that dietary Ca level of 2.5% (11.9 and 11.45 MJ ME/kg diet) is adequate to support egg production and eggshell quality in broiler breeder hens reared from 36 to 60 weeks of age. There were no beneficial effects of increasing Ca level from 2.5 to 3.5%. The Ca level of 2.5% is close to Ross Breeders recommended level of 2.8% (4-5 g). These results suggest that 2.5% Ca (3.8 g Ca/hen/day) is adequate to support egg production and to improve eggshell quality in broiler breeder hens. Egg production, egg mass, shell percentage and shell thickness declined but other parameters such as egg weight, egg contents, egg surface area, shell weight and SWUSA increased.

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EFFECT OF BREED, SEX AND SOURCE WITHIN BREED ON THE HEAMATOGICAL PARAMETERS OF THE NIGERIAN GOATS

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ABSTRACT: *Effect of breed, sex and source within breed, together with their interactions on the haematological parameters of Nigerian goats were studied using 81 goats (comprising 9 males and 18 females per breed), objective being to characterize and outline the differences and similarities between the breeds in blood parameters. The goats were derived from different geo-ecological zones in the country based on the areas of preponderance of each breed. The breeds studied were: the Sahel goat (SG), Red Sokoto goat (RSG), and West African Dwarf goat (WADG) and hematological values obtained per breed were: 22.52±1.48, 23.04±3.56, and 29.22±4.76 (%PCV); 7.52±0.50, 7.82±1.25 and 9.48±1.60 (g/dl Hb); 2.71±0.23, 3.09±0.64, and 4.10±0.42 (x10^{12}/l RBC); 11.94±1.10, 11.32±2.03 and 9.23±0.63 (x10^{9}cells/l WBC), and 83.22±1.67, 76.72±2.30 and 73.34±3.40 (x10^{6}/mm^{3} MCV), respectively. Significant differences (P<0.05) were observed between the breeds, but the platelets, MCH and leucocytes differential counts were similar (P>0.05) for all the breeds. The WADGs were superior to the RSGs and SGs in PCV, Hb, and RBC counts, but lower in WBC counts and MCV. The SGs were similar in most of the haematological profiles examined, irrespective of geo-ecological distance, indicating homogeneity of the breed. The sahelian goat breed also outscored other breeds in MCV, showing that the breed has greater propensity to transport oxygen and in situation occasioning oxygen starvation, the breed survives better. This explains the reason for the survival of the breed in arid and semi-arid zone. Gender has no effect on the MCV and the values of 83.22±1.67x10^{6}/mm^{3}, 76.72±2.30x10^{6}/mm^{3} and 73.34±3.40x10^{6}/mm^{3} were observed for the SG, RSG, and WADG, respectively.*

ORIGINAL ARTICLE

Keywords: *Indices, red Sokoto goat, Sahel goat, West African Dwarf goat, haematology*

INTRODUCTION

The major problem facing the third world countries is how to increase the biological value of their menu, and how to improve and maintain the productive potentials of their domestic livestock given adverse ecological and physiological constraints in the country. In Nigeria precisely, goat ranked second to poultry in terms of number among the farm livestock species, representing about 48.7 % of total domestic livestock (Oyenuga et al., 1974). So any meaningful progress in goat production entails general increase in meat production and consumption by the Nigerian populace. This suggests the relative importance of goats in livestock farming in Nigeria.

Three distinct groups of goats are found in Nigeria, and each has its unique utility. While the West African Dwarf Goat (WADG) is known for her resistance to trypanosomiasis and tolerance to harsh environmental conditions, the Red Sokoko breed (Maradi) is distinct for her excellent meat and milk yields, high quality skins, twinning ability and other characteristics. The multi-coloured Sahel breed is characterized by her high prolificacy, multiple births (twin, triplet and quadruplets) and good meat quality, but poor meat yield (Pagot, 1993).

Though a good number of researchers have carried out studies on some haematological indices of West African Dwarf goat (Daramola et al., 2003) and Red Sokoto goat (Olotu et al., 1998; Lazzaro, 2001; Tambuwal et al., 2002), little or no information exists on effect of breed, sex and geographical distance on these indices. This study was therefore designed provide further information on the haematological indices, such as RBC, Hb, PCV, WBC, Platelet counts and differential white blood cell counts of the Nigerian goat breeds, and to ascertain the effects of breed, sex

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and source within each breed on these parameters. In addition, information generated may be used as a guide for diagnosis, and treatment of many diseases associated with blood.

MATERIALS AND METHODS

The study was conducted at the Goat Research Unit of the Department of Animal Science and Fisheries, Faculty of Agriculture, Delta State University, Asaba Campus, Asaba, Delta State. Delta State falls within the humid tropics of Nigeria, and Asaba precisely lies between longitudes 6 ^{0}E and 8 ^{0}E, and between latitude 4 ^{0}N and 10 ^{0}N. It has a moderate climate with a very high temperature during the dry season and average rainfall during the rainy season.

Design of the Experiment

The experiment was conducted under a 2 x 3 factorial in a completely randomized design (CRD) to test the effects of sex, breed, and their interactions on the haematological parameters of the Nigerian indigenous goats. Sex was tested on two levels with unequal replicates of twenty-seven bucks and fifty-four does; while there were three breeds with twenty-seven goats for each breed. In addition, effect of source of goat within each breed on these parameters was tested using one-way classification.

The statistical model used:

$Y_{ijk} = \mu + B_i + S_j + (BS)_{ij} + €_{ijkl}$

Where:

Y_{ijk} -is the observed haematological index

μ -the population mean;

B_i – the effect of the breed, $_i$= 1, –, 3

S_j – the effect of $_j$th sex of the animal, $_j$=1, 2

$(BS)_{ij}$- is the interaction between breed and sex, and

$€_{ijkl}$ –is the error term associated with the observations.

Assumptions; error term is independently, identically & normally distributed, with zero mean and constant variance, that is, iind (0, δ^2).

Sources of the Experimental Animal

Each breed was randomly obtained from three different towns (at the rate of three males and six females from each town) based on area of its predominant in the country. The Sahelian goats (SGs) were procured from arid region, red Sokoto goats (RSGs) were sourced from semi arid region, while the West African Dwarf goats (WADGs) were obtained from rainforest zone where they are predominant. A total of nine goats (comprising three males and six females) were randomly selected from each location/ source in the region. Typical SG, RSG and WAD goat are shown in Figures 1-3, respectively.

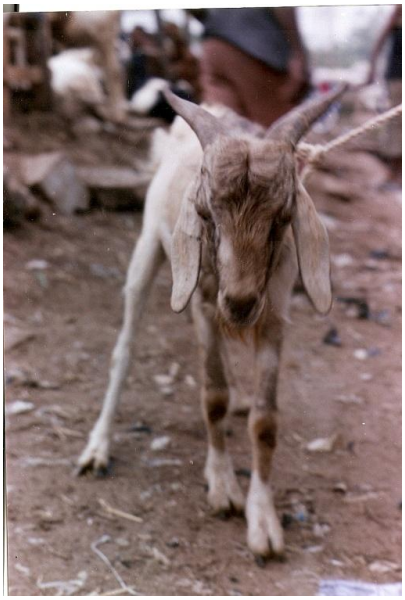

Fig. 1 - Typical Sahelian Buck

Fig. 2 - Typical RS Buck

Fig. 3 - Typical WAD Buck

Analytical Procedure

Sterilized syringe and hypodermic needles were used to collect 8ml of blood from the jugular vein of the animals. One syringe and needle were used per goat to avoid mixing-up or contaminations of blood samples. The

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samples collected were placed immediately on an anti-coagulant (EDTA) containing tube properly labelled for identification. The samples were shaken manually to prevent clotting. Sahlis's method was used to determine the haemoglobin concentration, while Formula citrate solution was used for RBC counts.

Turk's solution was used to dilute the blood sample prior to WBC counts, while 10% ammonium oxalate was used for platelet counts. For differential counts, a thin film of the blood sample was made on a grease free slide and allowed to air dry. It was stained with leishman's stain, diluted with buffer solution and washed off with water and allowed to dry. A drop of oil immersion was added and viewed for lymphocytes, neutrophils, eosinophils, basophils and monocytes.

Statistical Analysis

All the data collected were subjected to analysis of variance (ANOVA) appropriate for a 2x3 factorial in a CRD to test the effects of breed, sex and their interactions on the measured parameter. The differences between means were separated using Duncan's New Multiple Range Test (DNMRT). SPSS (2004) Statistical Package was used for data analysis.

RESULTS AND DISCUSSION

The values of PCV observed in this study (Table 1) is close to values recorded by Olotu et al. (1998), Lazzaro (2001) and Daramola et al. (2003), and within the percentage range of 22-38 % reported by Plumb (1999) and Tambuwal et al. (2002) for different breeds of goat. Breed differences ($P<0.05$) were observed in PCV percentages (Table 1). Pronounced breeds x sex interaction (Table 2) effects were observed in PCV only in the RSG breed where the bucks recorded higher ($P<0.05$) percentages than the does. Also, source x breed interaction effects (Table 3) on PCV were observed in the RSG and WADG. While the RSGs from Sokoto were higher in PCV than those from Gusau, the WADGs from Umuahia (South-East) were higher than those from Ughelli (South-South) and Akure (South-West). These discrepancies in PCV values as a result of breed, sex and location have been reported by Azab and Abdel (1999) and Tambuwal et al. (2002) for goats and Kaushish and Arora, (1977) in sheep. Also such variations have been reported by Orji et al. (1987).

Table 1 - Mean values of PCV, Hb, RBC, WBC and platelet counts for SG (B_1), RSG (B_2) and WADG (B_3) and with respect to gender

Parameter	B_1(SG)	B_2 (RSG)	B_3 (WADG)	Bucks	Does
PCV (%)	22.5±1.48^a	23.0±3.56^a	29.22±4.76^b	23.2±1.23^a	22.2±3.08^a
Hb (g/dl)	7.52±0.50^a	7.82±1.25^a	9.48± 1.60^b	8.44±1.03^a	8.19±1.66^a
RBC (10^{12}/l)	2.71±0.23^a	3.09±0.64^a	4.10±0.42^b	3.50±0.52^b	3.18±0.81^a
WBC (10^9/l)	11.9±1.10^b	11.3±2.03^b	9.23±0.63^a	10.2±1.21^a	11.7±1.25^b
Platelet Count x 10^9/l	139±6.49^a	146±17.10^a	146±9.19^a	141±9.72^a	145±14.60^a

*Means bearing different superscript letter on the same row for breeds and sex are significantly different ($P<0.05$)

Table 2 - Mean of PCV, Hb, RBC, WBC and platelet counts in bucks (males) and does (females) of each breed of goats

Breed	Sex	PCV %	Hb g/dl	RBC (x10^{12} /l)	WBC (x10^9/l)	Platelet count (10^9/l)
SG	Bucks	23.2±1.23^a	7.56±0.50^a	2.92±0.17^b	10.83±0.51^a	141±5.81^a
	Does	22.2±3.08^a	7.50±0.50^a	2.61±0.19^a	12.53±0.81^a	138±6.60^a
RSG	Bucks	25.1±1.91^b	8.75±0.97^b	3.62 ±0.27^b	10.86±1.31^a	143±12.20^a
	Does	21.0±3.74^a	7.33±1.11^a	2.82±0.60^a	11.55±2.27^a	147±22.0^a
WADG	Bucks	27.8±2.12^a	9.00±0.94^a	4.08±0.33^a	9.03±0.46^a	138±9.32^a
	Does	29.9±5.34^a	9.72±1.79^a	4.11±0.51^a	9.46±0.65^a	150±5.79^a

*Means bearing the different superscript letter along the same column within the same breed are significantly different ($P<0.05$).

The concentrations of Hb obtained in this study (Table 1) are comparable to the ranges reported by Tambuwal et al. (2002), Plumb (1999), Olotu et al. (1998) and Lazzaro (2001) and lower than the range 12-18 g/dl reported by Singh (2004) for man. The WADG has significantly higher ($P<0.05$) Hb concentration than the other breeds. But the SG and RSG are similar in the Hb concentrations. Sex has no pronounced effect (Table 1) on the Hb concentration, but when measured by breed (Table 2), it was discovered that the bucks of RSGs have higher ($P<0.05$) Hb values than their does. Source also exhibited significant effects on the Hb concentrations in the RSG and WADG. These discrepancies agreed with the documentations by various researchers in different livestock and man (Oduye and Adadevoh, 1976; Oduye and Otesile, 1977; Obi and Anosa, 1980; Singh, 2004). Generally increase in the Hb concentration is associated

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with greater ability to resist disease infection and low level is an indication of disease infection and poor nutrition (Cheesbrough 2004; Tambuwal et al., 2002).

The red blood cell counts obtained for the Nigerian goats were slightly lower than 5.70±0.10x10^{12}/l and 5.30±0.10x10^{12}/l reported by Tambuwal et al. (2002) for the WADG and RSG, respectively. The RBC count for the WADG falls within the border line of the range of 4.0-9.0 x10^{12}/l obtained by Olotu et al (1998), Plumb (1999), and Lazzaro (2001). The ranges observed for the RSG and SG breeds appear to be lower than the literature values (Olotu et al., 1998; Plumb 1999; Lazzaro 2001; Tambuwal et al., 2002). The low counts observed for the SG and RSG breeds could be as a result of physiological and environmental stress since the breeds were sourced from the north where they are predominance. Generally, the males have higher (P<0.05) RBC counts than the females. Except for the WADG, this is true even when the RBC count is determined on the basis of sex by breed (Table 3). Again, the source had a pronounced effect (P<0.05) on the RBC counts of the RSG and WADG. This agreed with the report of Cheesbrough (2004) who maintained that the RBC counts vary with age, gender, geographical location and health status of the animals.

Table 3. Mean values of PCV, Hb, RBC, WBC and platelet counts for source by breed

Breed	Source	WBC (x10^9/l)	Hb g/dl	RBC (x10^{12} /l)	PCV %	Platelet count (10^9/l)
SG	Maiduguri	12.2±1.34^a	7.67±0.47^a	2.81±0.21^a	22.8±1.37^a	135±4.91^a
	Potiskum	11.9±0.99^a	7.35±0.47^a	2.77±0.12^a	22.0±1.41^a	139±5.83^a
	Gumel	11.7±0.83^a	7.56±0.50^a	2.56±0.26^a	22.7±1.49^a	143±5.44^b
RSG	Sokoto	9.48±0.67^a	8.00±1.05^a	3.58±0.21^b	24.7±3.09^b	133±5.48^a
	Katsina	12.4±2.17^b	8.11±1.10^a	2.93±0.45^{ab}	22.9±2.69^{ab}	156±19.8^b
	Gusau	12.1±1.44^b	7.33±1.49^b	2.75±1.41^a	21.6±4.06^a	148±21.1^a
WADG	Umuahia	9.43±0.64^a	9.22±1.23^{ab}	4.31±0.51^b	28.4±3.72^b	148±7.51^a
	Ugheli	9.39±0.46^a	8.56±1.71^a	4.07±0.11^{ab}	26.8±5.69^a	148±5.14^a
	Akure	9.13±0.72^a	10.7±0.94^b	3.92±0.18^a	32.4±2.23^c	142±12.2^a

*Means bearing the different superscript letter along the same column within the same breed are significantly the different (P<0.05).

The WBC counts obtained in this study are statistically lower (P<0.05) in the WADG than in the other breeds (Table 1), but similar in the RSG and SG. The value obtained for the WADG is lower (P<0.05) than 13.5±0.8 x10^9/l reported by Tambuwal et al (2002); but higher than the value reported by Plumb (1999), and falls within the range of 7.5-15.8 x10^9/l documented by diverse researchers (Hunter 1996; Olotu et al 1998; Lazzaro 2001; Daramola et al 2003). Again, the values observed for the RSG (11.32±2.02 x10^9/l) and SG (11.94±1.10 x10^9/l) are close to 10.60±1.60 x10^9/l reported by Tambuwal et al (2002), and within the range given by some researchers (Olotu et al., 1998; Lazzaro 2001; Daramola et al., 2003). Generally, does are significantly higher (P<0.05) than bucks in the WBC counts, and the location of origin affected the WBC counts only in RSG breed. The RSGs from Sokoto are lower in WBC counts than those from Katsina and Gusau. This variation could be responsible for the concentration of the breed in Sokoto and its environs and as the breed disseminates to other parts, its inherent qualities continue to decline.

The platelet counts obtained in this study (Tables 1 and 2) are statistically uniform (P>0.05), irrespective of the breeds, sexes and sex by breed. The values are comparable to the literature values (Olotu et al., 1998; Plumb, 1999; Lazzaro, 2001; Daramola et al., 2003). Also the counts are very close the values reported for sheep (Kaushish and Arora, 1977; Oduye and Adadevoh, 1976) and for man (Graw, 2002; Singh, 2004). Significant locations differences (P<0.05) were observed (Table 3) in platelet counts in the SG and RSG breeds. While the SGs from Gumel are higher than the SGs from Maduguri and Potiskum, the RSGs from Katsina are higher than the RSGs from Sokoto and Gusau in platelet counts. Platelets play a critical role in the prevention of blood loss. At sites of minor blood vessel injury, platelets rapidly adhere to the exposed collagen, and then to one another to form a platelet plug which blocks the wound (Harper et al., 1977; Singh, 2004). According to them, thrombocytopenia (severe decrease in platelet counts) is associated with a bleeding tendency, while thrombocytosis (increase in platelet counts) may follow haemorrhage, Surgery or fracture of bone. But the platelet counts of all these goats fall within the normal range of 130-400 x10^9/l.

The percentage of lymphocyte, eosinophil, neutrophil, basophil and monocytes are similar (P>0.05) in the entire goat breeds studied (Table 4). That is, the percentage compositions of these individual cells in the leucocytes (WBC) are the same irrespective of the amount of the WBC in the breed. The values fall within the referral range (Hunter, 1996; Olotu et al., 1998; Plumb, 1999; Lazzaro, 2001; Tambuwal et al., 2002; Daramola et al., 2003). The bucks are higher (P<0.05) than the does in lymphocytes and eosinophil percentages, while the does are higher (P<0.05) in neutrophil counts (Table 4). Except for SGs, the same is true for sex by breed (Table 5); though all fall within the referral range. The higher WBC counts in the females are as a result of lower percentage of lymphocytes or more active response to the prevailing conditions. The SGs are similar (P>0.05) in all the leucocytes counts irrespective of geographical distance (Table 6); consequently, the breed is more homogenous compared to other breeds. The RSGs from Sokoto are higher in neutrophil counts, while those from Katsina are higher in lymphocytes, and those from Gusau

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are highest in eosinophil counts. These disparities are purely due to the location of origin. The sahelian goat breed recorded highest levels in MCV, so the sizes of their erythrocyte cells are larger than the cells in other breeds. Gender has no effect on the MCV. The values of MCV for the Nigerian goats (Table 4) were higher than 20- 60 $x10^6/mm^3$ reported by Tambuwal et al. (2002) and Plumb (1999) for different breeds of goat The MCH concentration is the same in the Nigerian goat breeds studied, and gender has no effect on the MCH concentration. The MCH values obtained ranged from 23.94-26.69 ug.

Discrepancies in most haematological indices between breeds observed in this study follow a similar trend in brood protein fraction and x-chromatin incidences among the breeds of Nigerian goats as reported by Okonkwo et al. (2010a) and Okonkwo et al. (2010b), respectively. Also, genetic diversity between Nigerian goat breeds has been unveiled by Adebambo et al. (2011).

Table 4 - Mean values of the MCV, MCH and differential counts of the Nigerian goats according to sex and breeds

Parameter	Bucks	Does	B_1 (SG)	B_2 (RSG)	B_3 (WADG)
Lymphocyte (%)	76.8±0.11^b	74.4±0.42^a	76.4±0.08^a	74.0±0.50^a	75.2±0.37^a
Eosinophil (%)	5.81±0.47^b	5.67±0.25^a	5.89±0.15^a	6.04±0.21^a	5.22±0.23^a
Neutrophil (%)	15.2±0.06^a	17.5±0.37^b	15.5±0.05^a	17.5±0.44^a	17.1±0.33^a
Basophil (%)	0.96±0.20^a	0.72±0.27^a	0.67±0.23^a	0.93±0.11^a	0.82±0.25^a
Monocyte (%)	1.44±0.10^a	1.69±0.19^a	1.63±0.06^a	1.37±0.26^a	1.82±0.14^a
MCV($x10^6/mm^3$)	73.6±14.21^a	79.8±15.3^a	83.2±1.67^b	76.7±2.30^a	73.3± 3.40^a
MCH (µg)	23.9±5.32^a	26.7±5.26^a	27.4±3.54^a	25.9±3.90^a	24.1±7.46^a

* Means bearing different superscript letter on the same row for sex and for breeds are significantly different (P<0.05).

Table 5 - Mean percentage values of the differential counts, and MCV and MCH values of bucks and does of the goat breeds

Parameter	B_1(SG)		B_2(RSG)		B_3(WADG)	
	S_1(M)	S_2(F)	S_1(M)	S_2(F)	S_1(M)	S_2(F)
Lymphocyte %	75..2±0.03^a	77.0±0.09^a	77.9±0.20^b	72.1±0.50^a	77.3±0.04^b	74.1±0.50^a
Eosinophil %	6.78±0.01^b	5.44±0.18^a	5.00±0.19^a	6.56±0.18^b	5.67±0.06 ^{b}	5.00±0.31^a
Neutrophil %	16.1±0.32^a	15.17±0.05^a	14.8±0.06^a	18.8±0.54^b	14.8±0.06^a	18.3±0.40^b
Basophil %	0.78±0.17^a	0.61±0.24^a	1.22±0.17^a	0.78±0.36^a	0.89±0.21^a	0.78±0.27^a
Monocyte %	1.56±0.04^a	1.67±0.07^a	1.11±0.15^a	1.50±0.30^a	1.67±0.04^a	1.89±0.19^a
MCV $x10^6/mm^3$	81.4±3.51^a	84.1±8.45^a	71.4±6.89^a	79.4±13.0^a	68.1±6.71^a	76.0±20.7^a
MCH(µg)	24.2±2.16^a	28.9±2.98^a	24.6±2.85^a	26.5±4.17^a	23.10±8.42^a	24.6±6.68^a

*Mean bearing different superscripts in the same row within the same breed are significantly different (P>0.05).

Table 6 - Mean values of MCV, MCH and differential counts for source by breed

Breed	Source	MCV $x(10^6/mm^3)$	MCH (µg)	LYMPH %	EOSINO %	NEUTR %	BASOPH %	MONOPH %
SG	Maduguri	81.8±6.58^a	27.4±2.54^a	77.1±0.07^a	5.67± 0.26 ^{a}	14.7±0.07^a	0.56±0.24^a	2.00±0.60^a
	Potiskum	79.5±2.35^a	26.6±2.00 ^{a}	76.6±0.14^a	5.67± 0.14^a	15.6±0.04^a	0.67±0.22^a	1.44±0.04^a
	Gumel	88.4±8.28 ^{a}	28.0±5.12^a	75.6±0.02 ^{a}	6.33±0.02^a	16.2±0.02^b	0.78±0.17^a	1.44±0.04^a
RSG	Sokoto	69.2 ±7.87^a	22.4±2.68^a	70.9±0.73^a	5.89±0.18^{ab}	21.0±0.76^c	1.00±0.59^a	1.11±0.07^a
	Katsina	80.4±13.0^b	28.0±3.72^b	78.0±0.06^b	5.44±0.14^a	13.8±0.01^a	0.67±0.31^a	1.89±0.08^a
	Gusau	80.6±10.6^b	27.2±2.53^b	73.2±0.38^a	6.78±0.29^b	17.7±0.13^b	1.11±0.27^a	1.11±0.27^a
WADG	Umuahia	67.3±12.8^a	21.8±4.20^a	73.0±0.13^a	7.33±0.14^b	17.6±0.15 ^{b}	0.56±0.32^a	1.67±0.07^a
	Ugheli	66.1±15.6^a	21.0±4.49^a	72.8±0.13^a	3.78±0.11^a	20.6±0.19 ^{a}	0.89±0.21^a	2.22±0.26^a
	Akure	86.6±16.3^b	29.5±9.21^b	79.7±0.41^b	4.56±0.11^a	13.2±0.15^a	1.00±0.13^a	1.56±0.07^a

*Means bearing different superscript letter along the same column in the same breed are significantly different (P<0.05).

CONCLUSION

WADGs are generally superior to RSG and SG breeds in erythrocyte parameters and have greater inherent ability to resist diseases and tolerate harsh tropical environment with high load of trypanosomiasis. Bucks are inherently superior to does in erythrocyte indices; therefore, can withstand more adverse conditions. The erythrocyte counts of Nigerian goat breeds are higher at the point or location where the individual breed predominates. The

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percentages of lymphocyte, eosinophil, neutrophil, basophil and monocyte are similar in all the three breeds of Nigerian goats studied. That is, the percentage compositions of these individual cells in the leucocytes (WBC) are the same irrespective of the amount of the WBC in the breed. The SG breed survives better than other breeds in semiarid and arid regions due to larger sizes of their red blood cells.

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EFFECT OF DIETARY CORN SILAGE REPLACEMENT WITH SORGHUM SILAGE ON PERFORMANCE AND FEED COST OF GROWING STEERS

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ABSTRACT: *This experiment conducted to assess effects of dietary corn silage (CS) replacement with sorghum silage (SS) on performance of growing Steers. 32 steers (182.3 ± 5 kg BW) randomly, in a CRD, allocated to 4 treatments of eight replicates. A diet of 60% hay (experimental part) plus 40% concentrate including barley, wheat bran, and soybean meal were fed for a period of 120 day. Hay included 40% of the same grass silage + 60% of different levels of SS and or CS, alone or in combination. SS was replaced with CS in steer rations with ratios of 0% (T1), 33% (T2), 66% (T3) and 100% (T4). Animals were weighed every week and information such as food intake (FI), daily weight gain (DWG) and food conversion ratio (FCR) were recorded in each replicate group and the body weight (BW) presented as a average of growth performance at the end of trial. Dietary CS replacement with SS significantly improved performance traits (P > 0.05), when SS was solely replaced in hay part of diet. The higher FI and lower FCR were observed in fattening bulls fed dietary group 4 (100% SS replaced in diet). Groups fed 33% SS (T2) did showed the higher DWG in compared to other groups. It is concluded that, the diet supplemented with 66 and or 100 % sorghum silage in 60% of hay portion, seem to be capable of improve performance accompanying with economic advantage in product prices.*

ORIGINAL ARTICLE

Key words: corn silage, sorghum silage, performance, carcass yield, Steer

INTRODUCTION

Sorghum grain is the principal grain used to finish cattle in some regions of the Iran and probably other Asian countries. It usually sells for less per pound than corn in Western countries such as the United States and can be a cheaper source of nutrients than corn for beef cattle rations but yet not be a better buy for cattle rations. Sorghum grain can be silage like corn grain. But the cost of sorghum silage, have no significant difference with the cost of corn silage.

Most studies have shown corn to have a higher feed value than sorghum grain for beef cattle. The protein and starch in sorghum grain are usually not as digestible as that in corn. Sorghum grain tends to vary more than corn in protein content and feed value because of cultural practices, soil fertility, and variety. Because, seed hardness of sorghum grain is high digestibility can be decreased that is linked to variety. Varieties with a floury type endosperm were higher in digestibility than those with a corneous-type (hard-type) endosperm.

Sorghum grain silage will not produce as many pounds of beef per acre as corn silage will on land suited to corn production. Tonnage of silage will be less per acre with sorghum grain and it will take more pounds of it to produce a pound of beef gain compared to corn silage. A study conducted by Al-Suwaiegh et al. (2002) documented that steers fed either corn or sorghum wet distillers grains, fed at 30% of the ration DM, had increased efficiency of

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gain. About varieties that are grown for grain yield, was averaged 1000 kg in hectare. Some researchers expressed forage sorghum yield between 50 to 60 tons per hectare (McCullough et al, 1981).

Hough et al (2002) reported that the rations containing corn silage reduced feed intake in heifers fattened compared with sorghum and silo due to reduced palatability of the diet because of the shape and appearance of corn silage. Moreover, it is likely to reduce feed intake in the presence of products resulting from fermentation in the silo that had a negative impact on eating diets based on corn silage (Tauqir et al, 2009). On the other hand, one of the causes of increased feed intake in diets based on sorghum silo can be related to high glucose in the stem and leaves of sorghum sugar by microorganisms that are used to reduce pH of silo below 4 and thus cause increasing the quality of the silo and on the other hand glucose of silo can increase palatability silo sorghum compared to corn silage (Ishin et al, 1985).

Therefore, an experiment was performed to assess effects of replacing corn silage with sorghum silage in diet on performance and feed cost of growing Steers.

MATERIAL AND METHODS

Farming and silo operations

After preparing the ground (any two pieces of land area 5.1 acre, located in 10 km West Gotvand city) to provide nutrients for plant growth than fertilizer N, P and K levels, respectively 250, 100 and 100 kilograms per hectare were used. After full growth and the emergence of plant seeds, forage sorghum and corn grain in dough stage using a chopper machine harvested and then accurately weighted using digital scale 60-ton and transmitted to animal farm and saved within separate silos with the dimensions of 3.1×5×15 then used the tractor to remove the remaining air inside the provender and the silos that quite compressed in order to prevent water penetration. In order to prepare laboratory samples, some of forage maize and sorghum into separate plastic bags 30 kilo grams (15 pieces) was silo.

Animal and housing

32 male calves of about 9 months old and the average weight of 182 kilograms were purchased from villages around the city of Dezful Shushtar and moved into research farm. Steers quarantined for 10 days for health operations, such as Colin afferent test, blood sampling for brucellosis disease and internal parasites, serve anti-parasitic drugs, disinfect livestock against ectoparasites and vaccination against common diseases in the area was conducted. In order to increase accuracy in measuring traits, 32 solo roofed status dimensions 4 × 2 meters with separate manger and watering with conditions almost identical in terms of light, air flow and other environmental factors were used. Calves in the four groups of eight head were randomly allocated in solitude positions. In order to habituate animals to test desired rations, the usual period for 15 days was applied. After weighing the animals, the main phase of the trial began for a period of 120 days. Weighing cattle performed at the beginning and end of each month and once after 12 hours of deprivation of food and water to obtain weight gain resulting in various stages of the trial course and also conducted for utilization from a new weight for the determination of lower DMI.

Feeding and performance calculating

Diets were formulated according to NRC (1989) related to cattle calves of the heavyweight strain and were fed to different experimental groups. Chemical composition of foods used in the experiment based on 100 percent dry matter is given in Table 1. Diets ingredients and composition is shown in Table 2. DMI was included from forage and concentrate in two parts and with a ratio of 55: 45. A diet of 60% hay (experimental part) plus 40% concentrate including barley, wheat bran, soybean meal, urea, calcium carbonate; mineral-vitamin premix and salt were fed for a period of 120 day. Hay included 40% of the same grass silage + 60% of different levels of SS and or CS, alone or in combination. SS was replaced with CS in steer rations with ratios of 0% (T1), 33% (T2), 66% (T3) and 100% (T4). Animals were weighed every week and information such as food intake (FI), daily weight gain (DWG) and food conversion ratio (FCR) were recorded in each replicate group and the body weight (BW) presented as a average of growth performance at the end of trial, then two steers per treatment were slaughtered after 12 hours dietary deprivation.

All diets in terms of energy and protein concentrations were similar. Dry matter intake, two meals daily in the morning and afternoon was weighed in a certain amount so that uniformly mixed were fed to animal, free choice. The next morning and before daily feeding, the remaining food of the manger was daily collected and weighed to calculating DMI. During the 15 day of habituate period and 120 days of the main trial period, clean and safe drinking water and rock salt lick blocks were provided for animals, *ad-libitum*.

Weighing calves once every month with a 12 hours retrieving food was done before every morning feeding and the results were calculated for each 30-day periods. Rate of weight gain per calf during each period with the weight difference between the beginning and end periods were determined. Average daily gain during each period by the following formula was calculated. Feed conversion ratio (FCR) by the amount of feed consumed per unit of live weight was calculated every 30 days as well as in total of the experimental period was marked by the following equation. FCR= Dry matter intake in each course (kg) / same period the amount of weight gain (kg).

Feed and live weight prices calculation

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Forage sorghum and corn planting costs for the silo and also food and diets prices per kilogram or per 100 percent dry matter (RLS) were calculated. Although, because of fluctuations in cost of one kilogram of live animal weight and one kilogram of feed intake (RLS) in the market is difficult to estimate the exact cost, the most common prices in different regions of Shushtar province were supplied and calculated. Unit cost is based on Iranian rial (IRR). For example for converting costs: 1 united state dollar (USD) in 2010 = 10500 ± 500 IRR.

Table 1 - Chemical composition of foods used in the experiment based on 100 percent dry matter

Diet ingredient	Dry matter	Protein	Cell wall	Cell wall without hemicellulose	Calcium	Phosphorus
corn silage	28.8	7.8	48.9	29.6	0.22	0.2
sorghum silage	25.9	7.38	54.6	38.7	0.21	0.15
Dried lucerne	89.2	17.2	43	32.3	1.4	0.27
Barley	90.3	10.8	22	9	0.05	0.3
Wheat straw	89.5	15.25	43.2	17.1	0.12	1.1
Soybean meal	89.3	42	23.1	12	0.35	0.63
Urea	100	280	_____	_____	_____	_____
Calcium carbonate	100	_____	_____	_____	39.39	_____
Vitamin & Mineral supplement	100	_____	_____	_____	_____	_____
Salt	100	_____	_____	_____	_____	_____

Table 2 - Composition and components used in diets fed tested buffalo calves

Diet ingredient (%)	Treatments			
	1	2	3	4
Corn silage	0.0	7.80	48.9	29.6
Sorghum silage	40.0	7.38	54.6	38.7
Dried Lucerne	15.0	17.2	43.0	32.3
Barley	55.0	55.0	55.0	55
Wheat straw	21.0	21.0	21.0	21
Soybean meal	15.3	15.3	15.3	15.3
Urea	7.2	7.2	7.2	7.2
Calcium carbonate	0.4	0.4	0.4	0.4
Vitamin & Mineral supplement	0.4	0.4	0.4	0.4
Salt	0.3	0.3	0.3	0.3
Diet ingredient	0.4	0.4	0.4	0.4
Concentrate (kg)	45.0	45.0	45.0	45.0
Calculated nutrient content				
Metabolizable energy (Mcal/kg DM)	2.42	2.4	2.37	2.35
Crude protein (%)	14.37	14.32	14.26	14.21
Dry matter (%)	65.48	65.10	64.69	64.31
NDF (%)	38.91	39.70	40.45	41.21
ADF (%)	22.25	23.36	24.57	25.79
Ash	5.90	6.07	6.23	6.39
Calcium (%)	0.50	0.55	0.60	0.60
Phosphorous (%)	0.40	0.39	0.38	0.38

Statistical Analysis

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The data obtained from research using Excel software were calculated. All data by statistical software SAS (2001) using the following statistical model analysis (Yij = μ + Ti + εij) were compared. Yij = view about the treatment i and replicate j, μ = population mean, Ti = fixed effect of treatment I, εij = experimental error effect.
Effect of initial weight as Covariance in the model considered for final weight traits according to below statistical model: FWij = μ + Ti + b (IWij) + εij. FWij = (final weight) related to treatments i and replicate j, μ = population mean, Ti = fixed effect of treatment i, b (IWij) = initial weight of treatments i and replicate j, εij = experimental error effect. For significant differences (P < 0.05), means were compared by the Duncan test.

RESULTS AND DISCUSSION

Daily dry matter intake, Daily weight gain, Feed conversion ratio (FCR)

Comparison of results related to effect of substituting different levels of sorghum with corn silage to increase the final weight of steers is given in Table 3. Results showed that replacement of sorghum silage with corn silage significantly decreased the final weight (P<0.05), so that treatment 4 (contains maximum sorghum silage) had lowest live weight with a significant difference compared to other treatments. Comparison of the mean showed that replacing corn silage with sorghum silage significantly affected feed intake (P<0.05), so that highest FI was observed in treatment 4.

Results showed that during the first month of trial, rate of overweight group 2 significantly higher than the first and fourth groups (P<0.05). During the first month of study, calves fed containing diets sorghum silage compared with the control diets had greater daily weight gain. In the second month of feeding replacement diets to animals, highest weight gain rate was numerically related to T1 and T3 however between both treatments the difference was not significant. During the third month overweight rates in T2 was significantly higher than T3 and T4. The lowest weight gain was related to T4. Results during the fourth month of trial were almost similar results of third month. Overall the highest and lowest weight gain was related to the second and fourth treatments, respectively. From the results in this study it is detected that replacing corn silage with sorghum silage up to 66% could not significantly affected the average daily weight gain of calves, but when replacement level reached to 100 %, significant decrease in WG was observed.

Results related to the effect of substituting different levels of sorghum with corn silage on feed conversion ratio of fattened calves tested in different months is given in Table 3. During the first month, the highest feed conversion ratio was observed in treatment 4 (6.66) that except T1 significantly differenced with values of T2 and T3 (P<0.05). The lowest FCR (5.93) observed in group 3 (animals fed 66% CS) with a significant difference with groups 1 and 4. In throughout trial period (four months) the highest FCR in a numerical fashion was related to the treatment 4 (100% CS) and the lowest FCR was related to T2.

All treatments received different levels of corn silage (treatments 1, 2 and 3) were not significantly different, but had significant difference in comparison with treatment 4 (P<0.05).

Performance

According to Table 3, although increasing replacement of sorghum silage with corn silage in steer diets up to 66% (T3) had not significant effect on body weight but dietary SS replacement by 100% level (T4) significantly increased DMI and decreased FCR accompanying a significant decline in body weight.

The cause of reduced feed intake can be inverse correlation NDF concentration of diets and feed intake noted. Previous research conducted with sorghum and corn silage silo showed the highest negative correlation between NDF concentration and dietary intake related to rations based on corn silage is (Nichols et al, 1998). Hough et al (2002) reported that the rations containing corn silage reduced feed intake in heifers fattened compared with sorghum and silo due to reducing palatability of the diet because of the shape and appearance of corn silage. Moreover, it is likely to reduce feed intake in the presence of products resulting from fermentation in the silo that had a negative impact on eating diets based on corn silage (Tauqir et al, 2009).

On the other hand, one of the causes of increased feed intake in diets based on sorghum silo can be related to high glucose in the stem and leaves of sorghum sugar by microorganisms that are used to reduce pH of silo below 4 and thus cause increasing the quality of the silo and on the other hand glucose of silo can increase palatability silo sorghum compared to corn silage (Ishin et al, 1985). The difference between the results of different studies can be due to species and breed of animal experiments, physiological maturity stage, the physiological form and amount of nutrients, conditions and testing different varieties and other environmental factors is used (Manhanta and Pachauri, 2004; Nichols et al, 1998).

High fiber according to the silo sorghum is expected to increase its level in the diet increased feed intake and thus weight gain is increasing, but factors such as high fiber, lignin and tannin in sorghum increased silo food passage rate of gastrointestinal tract and digestibility are reduced (Gnsrm, 1373), which eventually would be reduced daily gain in treatments of sorghum silage (t3 and T4).

On the other hand increased their feed intake increases the passage rate of gastrointestinal digestion materials by microorganisms thus less time to have a material impact on the result of reduced digestibility and consequently also reduced weight gain (rejection, 1386). Mole and Waterman (1987) on 38 animal research conducted, which was determined that high levels of tannin (20-10 percent) decreased the growth rate of sheep was due to reduced digestibility and thus weight gain reduced. Whatever digestibility of dry matter is less, the amount of

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material absorbed from digestive canal will less, and excretion of materials from gastrointestinal tract will further that this can affect daily weight gain and subsequently feed conversion ratio (Cunh, 2001). DM digestibility is dependent to content of lignin and crude fiber. There is evidence that the strong connections between lignin and many plant polysaccharides and cell wall proteins (lignocellulosic complex) prevents from digesting carbohydrates or reduces the rate of digestion (Cunh, 2001).

Table 3 - Effect of replacing different levels of corn silage with sorghum silage on performance (mean ± standard error)

Parameters	Treatments			
	1	2	3	4
Average initial weight	185.40 ± 3.21[a]	178.00 ± 2.91[a]	187.20 ± 4.20[a]	177.80 ± 3.80[a]
Average final weight	302.41±4.86[a]	301.7 ± 5.43[a]	303.88 ± 5.25[a]	290.87 ± 4.10[b]
Feed intake				
First Month	5.61 ± 0.75[b]	5.76 ± 0.96[ab]	5.36 ± 0.10[b]	6.01 ± 0.10[a]
Second month	6.11 ± 0.85[c]	6.44 ± 0.11[b]	6.54 ± 0.11[ab]	6.84 ± 0.11[a]
Third Month	6.86 ± 0.99[b]	7.12 ± 0.12[ab]	6.78 ± 0.12[b]	7.25 ± 0.11[a]
Fourth Month	7.42 ± 0.11[b]	7.79 ± 0.13[ab]	7.45 ± 0.12[b]	8.03 ± 0.11[a]
Total Volume	6.50 ± 0.92[b]	6.76 ± 0.11[ab]	6.62 ± 0.11[b]	7.30 ± 0.11[a]
Daily weight gain				
First Month	0.88 ± 0.019[c]	0.97 ± 0.02[a]	0.95 ± 0.02[ab]	0.90 ± 0.01[bc]
Second month	0.96 ± 0.018	0.94 ± 0.03	0.92 ± 0.03	0.95 ± 0.02
Third Month	1.01 ± 0.02[ab]	1.07 ± 0.03[a]	0.98 ± 0.02[ab]	0.93 ± 0.02[c]
Fourth Month	1.05 ± 0.03[ab]	1.10 ± 0.02[a]	1.30 ± 0.02[ab]	0.99 ± 0.02[bc]
Total Volume	0.97 ± 0.02[ab]	1.02 ± 0.02[a]	0.97 ± 0.02[ab]	0.94 ± 0.01[b]
g feed/g gain				
First Month	6.41 ± 0.10[a]	5.95 ± 0.11[b]	5.93 ± 0.14[b]	6.66 ± 0.10[a]
Second month	6.36 ± 0.06[b]	6.89 ± 0.21[a]	7.15 ± 0.20[a]	7.20 ± 0.11[a]
Third Month	6.75 ± 0.05[b]	6.67 ± 0.15[b]	7.02 ± 0.13[b]	7.80 ± 0.17[a]
Fourth Month	7.80 ± 0.10[b]	7.03 ± 0.10[b]	7.17 ± 0.09[b]	8.13 ± 0.20[a]
Total Volume	6.65 ± 0.06[b]	6.63 ± 0.12[b]	6.82 ± 0.12[b]	7.45 ± 0.10[a]

[ad,] Values in the same row and variable with no common superscript differ significantly (P<0.05).

In general, older animals tend to have more fat and saves since carcass fat than protein with small amounts of water, pounds of fat stored on a supply of stored energy than one kilogram of dietary protein, more expensive and the need to consume more food and feed in ruminants on the other hand increased the absolute weight increases, so can say with weight gain in ruminant feed conversion ratio is worse (Fazaeli et al, 2006). Differences in food conversion ratio between different experiments indicate that several factors such as age and breed animals, initial weight, forage: concentrate ratio, type and quality of food rations and other environmental factors such as temperature can be over affect feed conversion ratio (Fazaeli et al, 2006).

Comparison of production costs

Project costs related to food and diets prices per kilogram dry matter is shown in Table 4 and Forage sorghum and corn planting costs for the silo is shown in Table 5. The results showed that levels of income per acre of corn compared with sorghum was more, but in contrast, the cost per ton of the produced silage for sorghum forage was 146,413 rials that in compared to 174,781 rials of corn was lower. Price per kg DMI (dry matter based) and cost per kg live weight of livestock not significantly changed between treatments (Table 6), so that with replacing SS in diet costs of one kg of feed decreased and cost of one kg live weight increased.

Sorghum and corn production costs

According to Table 5 it is indicated that the level of income per hectare of corn compared with sorghum was more that could be due to higher product and the high price of corn forage compared with sorghum. Dumler (2008) with study on sorghum was concluded that the amount of revenue and costs of production significantly depends on the product value and stated that with increasing production of products, production costs per unit was reduced and the resulting revenue increase. On the other hand the cost of producing one ton of forage of sorghum compared with

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corn is much lower, which is due to use smaller amounts of seed (5.4 vs. 30 kg, respectively), fertilizer, water, manpower etc. in cultivation of sorghum than maize. Less use of these inputs lowers costs and thus bring about reduce the production cost of one ton of sorghum forage in compared to maize.
In experimental diets with increasing levels of sorghum silage in replace with corn silage, price per kg of DMI is reduced due to lower prices of sorghum silage than corn silage (Table 6).

Table 4 - Food and diets prices per kilogram, or per 100 percent dry matter (RLS)

Diet ingredient	Per kilogram	Per 100%DM	1	2	3	4
corn silage	400	1544	652.7	435.71	217	0
sorghum silage	470	1631.9	0	205.3	412.2	617.6
Dried lucerne	2000	2242	336.3	336.3	336.3	336.3
Barley	2200	2436	511.5	511.5	511.5	511.5
Wheat straw	1700	3899	290.5	290.5	290.5	290.5
Soybean meal	4500	5039	362.8	362.8	362.8	362.8
Urea	500	500	2	2	2	2
Calcium carbonate	500	500	2	2	2	2
Vitamin & Mineral supplement	700	7000	21	21	21	21
Salt	400	400	1.6	1.6	1.6	1.6

Table 5 - Forage sorghum and corn planting costs for the silo

Cost	Sorghum (per hectare)	Corn (per hectare)
Product per hectare (tons)	56.88	61.7
Price per ton product (RLS)	400000	470000
Income per hectare (RLS)	22752000	2899000
Seed	135000	900000
Herbicide	142000	142000
Fertilization	217000	277000
Expenses machinery	3358960	3860000
Water cost	250000	450000
Human resources	1425000	2355000
Ground rent	2000000	2000000
Miscellaneous	800000	800000
Total costs	8327960	10784000
Cost per ton silage (total cost / value of product)	146413	174781

Table 6 - Price per kg DMI (dry matter based) and cost per kg live weight of livestock

Parameters	1	2	3	4
Price of one kilogram of feed intake (RLS)	2180.4	2168.71	2159.9	2147.3
Cost of one kilogram of live animal weight (RLS)	14477.8	14378.5	14708.9	15975.9

Therefore, because of increase the replacement percentage of SS with CS, price per kg DMI also reduced. Cost per kilogram of live animal weight in parallel with percent substitution SS with CS has upward path due to the increase in feed conversion ratio. Highest price of a kilogram of animal body weight is related to T4 (100% SS) is due to higher

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feed conversion ratio compared to other treatments. Lowest cost of a kilogram live weight of cattle is belonging to the second group (33% SS) because of its low feed conversion.

CONCLUSION

From the results of present study, the higher feed intake and lower feed conversion ration were observed in fattening bulls fed dietary group 4 (100% SS replaced in diet). Groups fed 33% SS (T2) did showed the higher DWG in compared to other groups. Considering to entire cost per kg SS and CS, cost of one kg of food intake had a descended path from T1 to T4. However, price of one kilogram of FI not significantly decreased, cost of one kg of cattle live weight in SS group increased slightly, due to higher FCR of sorghum silage. As a consequence, the diet supplemented with 66 and or 100% sorghum silage in 60% of hay portion, seem to be capable of improve performance.

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EFFECT OF WEANER BODY WEIGHT ON GROWTH TRAITS OF RABBITS

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ABSTRACT: *Data from growth parameters and production traits of 108 ten-week old male rabbits comprising Newzealand white, Dutch and Chinchilla breeds collected over a period of 16 weeks were analysed in a 2- factor factorial in a randomised complete block (RCBD) to determine their interrelationships and response to age and weaner body weight groupings as a step towards employing them in selection and breeding programme and predicting live body weight. Individual breeds were grouped into three categories of body weights of low (LBW), medium (MBW), and high (HBW), depending on the body weight range of each breed. The linear body parameters were characterised using Body weight (BW), Heart girth (HG), Shoulder to tail drop, Head to shoulder, Ear length and Tail length, while the Production traits studied were average Daily feed intake, Feed conversion efficiency and mortality. Results of the analysis evinced significant (P<0.01) effect of breed-body weight interaction on the Production traits and linear body parameters studied. High weaner body weight Chinchilla consumed more feed; converted feed to meat more efficiently, gained weight more rapidly and recorded no mortality. Regression and correlation studies revealed that any one of the linear body traits could predict the rabbit body, weight at 20 weeks of age. Trait combination revealed that Ear length Vs Height at withers (R2-0.944), Ear length Vs Heart girth (R2-0.969) and Heart girth (R2= 0.935) best contributed to the total variability in body weight of Newzealand white Dutch and chinchilla respectively.*

Keywords: *Weaner, body weight, growth traits and rabbits*

ORIGINAL ARTICLE

INTRODUCTION

The problem of inadequate supply of animal protein from conventional sources like sheep, goat and chicken has led to the search for other sources of animal protein. The rabbit has been seen to be suitable in this regard. This is attributed largely to the rabbit's high rate of reproduction, early maturity, rapid growth rate, efficient food utilization and high quality nutritive value (Chineke 1996). Improvement of economic characters in animals requires estimates of genetic, environmental and phenotypic parameters for the various traits of interest. In order to achieve this goal, proper measurement of growth traits on important economic characters is required.

Body weight is regarded as a function of framework or size of the animal and its condition (Philip 1990). Variation in body weight within a flock can be attributed to genetic variation and environmental factors that impinge on individual (Ayorinde and Oke 1995). Body weight is known to be moderately to highly heritable and hence the selection of heavier individuals in a population should result in genetic improvement of the traits. Post weaning viability at different ages up to 16 weeks of age is significant in the economics of rabbit production. It has an important influence on the number of marketable rabbits and can be used in selection programmes for increasing productive efficiency (Afifi and Emara 1988). The study was therefore carried out to determine the effect of weaner body weight on production traits of rabbits as a step towards employing them as a correction factors in body weight estimation for selection purposes.

MATERIAL AND METHODS

The experiment was carried out at the Rabbitry unit of Michael Okpara University of Agriculture, Umudike Abia State, Nigeria. The university lies within the tropical rainforest belt of Nigeria. The area is located, on latitude 05^0 29N, Longitude 07^033E and altitude of 122m above sea level. The daily mean temperature is 24.2^{0c}. The fluctuation is usually of wide variation, especially during the day although the nights are generally cooler. The zone

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has a maximum daily temperature of about 33^{0}c. The annual rainfall is about 2100 to 2500mm. The mean relative humidity is about 85%, although, daily values are usually liable to wide variation.

One hundred and eight 10-week old male rabbits of different breeds raised intensively were used for the study. The breeds were Newzealand white, Dutch and chinchilla. The three breeds were obtained from a total of 54 pure breeding does and 12 adult bucks, consisting of 18 New Zealand white, 16 Chinchilla, 20 Dutch and 4 bucks each of the three breed respectively and were used to produce 108 kits from 4 litters. Individual breeds were grouped into three categories of body weights of low (LBW), medium (MBW) and high (HBW0, depending on the body weight range of each breed. The rabbits were reared in individual pens, fed a concentrate ration of 19 percent crude protein, given free choice and supplemented with Panicum maximum and Centrosema pubescens. Fresh clean water was also supplied regularly.

Data collection and Statistical analysis

Records of feed intake, average daily gain and mortality were kept. Body linear measurements were determined using a tape rule. The ear length was taken as the length from the base of the ear to the tip,
Heart girth-circumstances of the body measured behind the forelimbs round the chest, Height at withers –vertical height at resting position, Head to shoulder-horizontal joining head to shoulder, Shoulder to tail drop- length from shoulder to the base of the tail, Tail length-length from shoulder to the tail end.

The experimental design adopted was 2-factor factorial in randomised complete block (RCBD). The factors were body weight (low, medium, high) and the genotype (Newzealand white, Dutch and chinchilla), while the age of the rabbits served as the block. The data obtained were subjected to analysis of variance using the model

$$Y_{ijkl} = \mu + A + G_j + W_k + (GW)_{jk} + e_{ijke}$$

Where Y_{ijkl} a single observation

A_i = effect of the i^{th} age
G_j = effect of j^{th} genotype (Chinchilla, Dutch, New Zealand white)
W_k = effect of the j^{th}, k^{th}/genotype
μ = overall mean
$e_{ijk}l$ = random error

Pearson's correlation among the various body measurements were estimated and significant test carried out. Simple regression of the body traits on age was conducted using the model

$y = a + b_{xi} + e_i$
a = constant
b = regression coefficient i.e. change in the dependent variable (body trait) resulting from a unit change in the independent variable X_1 (age)
e_i, = random error

A stepwise regression analysis was carried out, using the model.

$$a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 - + b_nx_n — + e_i$$

where

BW = body weight
a = constant
b,b,2 – b_nX_n = regression coefficient for traits $X_1X_2—X_n$ respectively
e = error

The SPSS (1999) package was used for all statistical analysis.

RESULT AND DISCUSSION

The daily intake, feed efficiency, average daily body weight gain was significantly ($P<0.01$) different for the various genotype-body weight class interaction (Table 1). This result explains that breed-type-body weight class differences have significant effect on feed intake average daily body weight gain and feed efficiency. Thus, the rabbit of low body weight consumed the least daily, gained weight less readily and least converted feed to meat efficiently compared to the rabbit of higher body weight in all the breeds. The result also shows that the chinchilla gave the highest values for these traits. This could be explained by the fact that it is a heavy breed and thus benefits from the advantages posed by the established positive relationship between body weight and feed intake, feed efficiency and average daily gain. Buttressing this assertion, Ayorinde and Oke (1995), reported that the metabolic size of the animal is an important function of the animal appetite and therefore influences the total amount of feed consumed by the animal, thus the larger the animal the higher the feed intake. Burn and Ouhaoun (1981), confirmed that the Fleming giant crossbred litters of rabbits which consumed more feed, gained more rapidly and utilized feed more efficiently and weighted more at 70-day market weight when compared to the Newzealand white sired litters which consumed less feed and thus achieved slower gain, converted feed to gain less efficiently and consequent had the

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numerically lowest mean market weight at the same age. Ayorinde (1997) observed that the initially higher pre-wearing body weights of the Dutch and Newzealand white gave them an advantage to 18 weeks of age.

Table 1 - Means of the FI, ADG, FE and MORT for the various genotype-body weight interactions

Genotype	Body weight class	Daily fed intake (g)	Average Daily weight Gain (g)	Feed efficiency	Mortality %
Newzealand White	LBW	41.136±0.907[a]	8.619±0.310[a]	4.842±0.44[c]	3.3
	MBW	52.789±0.907[b]	12.771±0.310[b]	4.141±0.044[b]	–
	HBW	54.809±0.907[c]	13.579±0.310[c]	4.052±0.044[a]	–
Dutch	LBW	53.150±0.907[a]	12.326±0.310[a]	4.344±0.044[c]	–
	MBW	60.244±0907[b]	14.857±0.310[b]	4.064±0.044[b]	–
	HBW	66.006±0.907[c]	17.365±0.310[c]	3.806±0.044[a]	–
Chinchilla	LBW	54.561±0.907[a]	13.619±0.310[a]	4.09710.044[c]	–
	MBW	66.944±00.907[b]	17.793+0.310[b]	3.780±0.044[b]	–
	HBW	80.978±1.283[c]	22.857±0.438[c]	3.553±0.063[a]	–

[a,b,c] means in the same column and with different subscripts are significantly different at P<0.01. MBW: Medium body weight. LBW: Low body weight. HBW: High body weight

The finding in this work recorded 3.3% mortality for the low body weight Newzealand white. This mortality could be explained by the fact that the rabbit possessed low body weight and thus consumed less feed daily, gained less rapidly and consequently was more susceptible to adverse environmental condition like infection.

Thus, the heavy breeds and weaners of higher body weights are most recommend for selection and breeding. The effect of the genotype-body weight class interaction on the linear body measurement was significantly (P< 0.01) different as shown in table 2. The findings in this work explains that rabbits that possessed initially low body weight did not perform as much as those of initially high body weight in terms of final body weight and linear body traits. The result also revealed that the heavy breed (chinchilla) recorded the highest values for the final body weight and linear body traits at 20 weeks of age, while the Newzealand white (LBW) recorded the lowest values for the final body weight and linear trait at the same age. This finding corroborates the work of Thomas and Nandakumar (2001) who asserted that the chinchilla, which possessed the highest body weight, had the highest values of the linear body traits. Explaining this, Roberts, (1963), added that there is a moment-by-moment increase on percentage basis on the existing body weight of the individual animal. This will mean that a positive correlation is existed between body weight and linear body measurement, thus selection of any of the body measurements for improvement would mean a concomitant improvement on the body weight. This suggests that choosing rabbits of increased linear body measurement would imply choosing for heavier body weights.

Results show that the correlation among linear measurement in Newzealand white rabbits is positively very high and significant (P<0.01) (Table 3). The correlation matrix showed live weight was significantly (P<0.01) and positively correlated with body length (0.818) tail length (0.865), head to shoulder (0.897), Height at wither (0.903), heart at girth (0.934) and ear length (0.958). This high correlation between live weight and heart girth has long been recognised in livestock and has been reported by Johanson and Hildeman (1954) who noticed a correlation of 0.97. From the results, ear length proved the best indicator of body size for the Newzealand white. The interrelationship among the linear traits reveals that body length (shoulder to tail drop) was most correlated to head to shoulder. This means that selection for improvement in the head to shoulder would mean increased body length and subsequent body size increase. This also implies that absolute length and head to shoulder are complementary.

The results also show that correlation among linear measurement and body weight in the Dutch were positive, high and significant (P< 0.01) (Table 4). The work of Lawrence and Fowler (1997) supported this assertion. Specifically, the matrix indicates the live weight was significantly (P<0.01) and positively correlated with heart girth (0.797), head to shoulder (0.872), tail length (0.898), body length (0.900), height at withers (0.947) and ear length (0.983). The result depicts ear length as the best predictor of body size. The result of the interrelationship among the linear traits show that body length and head to shoulder are most correlated (0.959). This would mean that improvement on head to shoulder would most increase body length. However, this disagree with the finding of Tiamiyu et al.,(2001) who observed that body length and heart girth were most correlated. (0.95). These deviation may be explained by the assertions of Ibe and Ezekwe (1994), who maintained that different linear body traits measurement would be required to quantify body shape and size in different breeds and under different conditions.

The correlation between body size and linear trait measurements in the chinchilla were positive, high and significant (P<0.01) (Table 5). The matrix reveals that body weight is significantly (P<0.01) and positively correlated with head to shoulder (0.888), body length (0.988), height at withers (0.902) tail length (0.92) ear length (0.952) and heart at girth (0.967).

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Table 2 - Means of the body linear traits for the genotype-body weight interaction

Genotype Trait	Body weight class	Body weight (g)	Tail length (cm)	Heart at girth (cm)	Ear length (cm)	Head to shoulder (cm)	Height at withers (cm)	Shoulder to tail drop (cm)
Newzealand	LBW	1105.90±29.847[a]	8.32±0.149[a]	19.353±0.114[a]	9.325±0.074[a]	8.88±0.130[a]	11.96±0.078[a]	20.497±0.240[a]
	MBW	1467.75±29.847[b]	11.57±0.149[b]	21.28±0.114[b]	10.016±0.074[b]	10.70±0.130[b]	12.69±0.078[b]	23.284±0.240[b]
	HBW	1517.75±29.847[c]	12.24±0.149[c]	21.81±0.114[c]	10.524±0.074[c]	11.66±0.130[c]	13.34±0.078[c]	24.890±.240[c]
Dutch	LBW	1438.70±29.847[a]	8.57±0.149[a]	19.02±0.114[a]	9.932±0.074[a]	8.19±0.130[a]	12.82±0.078[a]	23.704±0.240[a]
	MBW	1657.35±29.847[b]	9.67±0.149[b]	22.43±0.114[b]	10.449±0.074[b]	8.95±0.130[b]	13.22±0.078[b]	24.785±0.240[b]
	HBW	1865.15±29.847[c]	10.58±0.149[c]	23.43±0.114[c]	10.805±0.074[c]	10.51±0.130[c]	13.82±0.078[c]	24.487±0.240[c]
Chinchilla	LBW	1488.05±29.847[a]	7.21±0.149[a]	21.55±0.114[a]	9.785±0.074[a]	7.91±0.130[a]	13.20±0.078[a]	21.470±0.240[a]
	MBW	1873.35±29.847[b]	8.99±0.149[b]	23.23±0.114[b]	10.404±0.074[b]	10.43±0.130[b]	14.32±0.078[b]	24.668±0.240[b]
	HBW	2304.50±42.210[c]	10.97±0.211[c]	25.11±0.162[c]	11.752±0.074[c]	11.53±0.184[c]	15.08±0.111[c]	28.970±0.339[c]

[a,b,c] means in the same column and with different subscripts are significantly different at P<0.01.

Table 3 - Coefficients of the correlations between characters in Newzealand White

Parameters	Body Weight	Tail length	Heart girth	Ear length	Head to shoulder	Height at Withers
Tail length	0.865**					
Heart girth	0.934**	0.935**				
Ear length	0.95**	0.916**	0.957**			
Head to shoulder	0.897**	0.973**	0.918**	0.958**		
Height at withers	0.903**	0.941**	0.930**	0.979**	0.978**	
Shoulder to tail	0.818**	0.961**	0.850	0.903**	0.983**	0.950**

** Significantly different at P<0.01.

Table 4 - Coefficient of correlation between characters in Dutch

Parameters	Body Weight	Tail length	Heart girth	Ear length	Head to shoulder	Height at withers
Tail length	0.895**					
Heart girth	0.797**	0.907**				
Ear length	0.983**	0.926**	0.838**			
Head to shoulder	0.872**	0.956**	0.879**	0.882**		
Height at withers	0.947**	0.942**	0.871**	0.956**	0.952**	
Shoulder to tail	0.900**	0.950**	0.838**	0.916**	0.959**	0.931**

** Significantly different at P<0.01.

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Table 5 - Coefficient of correlation between characters in Chinchilla

Parameters	Body Weight	Tail length	Heart girth	Ear length	Head to shoulder	Height at withers
Tail length	0.921**					
Heart girth	0.967**	0.966**				
Ear length	0.952**	0.916**	0.972**			
Head to shoulder	0.888**	0.967**	0.928**	0.841**		
Height at withers	0.902**	0.952**	0.956**	0.917**	0.925**	
Shoulder to tail	0.988**	0.992**	0.941**	0.888**	0.955**	0.913**

** Significantly different at P<0.01.

From the foregoing heart girth is the best predictor of body size in chinchilla. This assertion corroborates the findings of Tiamiyu et al (2000) and Johanson and Hilderman (1954). The results of interrelationship among the linear body traits show that body length and tail length (0.992) are best correlated. This indicates that absolute body length and tail length are complementary and this means that selection of heavy breed rabbits that are long in body and tail may be practicable. These features are thus indicator of good conformation.

The magnitude of the coefficient of determination (R2) for each parameter in the regression equation show the relative contribution of each body measurement to the body weights of rabbits at 20 weeks of age. The results show that the coefficient of regression of the body traits on age is positive and significant for all the breeds (P<0.01). This is confirmed by the work of Chineke et al 2000, who reported a consisted increase in body measurements of rabbits with age. The three breeds revealed ear length as the best regressed on age and thus contributed most to body weight of the rabbits at 20 weeks of age. The ear length contributed 38.9, 59.6, and 42.7% to the body weight of the New Zealand white Dutch, and Chinchilla respectively at 20 weeks of age. However, heart girth contributed second best to body weight in New Zealand white (30.1%) and chinchilla (36.9%) at the same age. The stepwise regression of body weight on the various body linear traits (Tables 6 and 7) revealed that in the New Zealand white, 94.9% of body weight is attributable to ear length, height at withers and tail length at 20 weeks of age, while only 94.5% of body weight is attributed to ear length and height at withers. The combination of traits that gives the highest R^2 value depicts the best predictor of body weight. However, since both combinations contribute approximately the same (95%) to body weight variability, it would be most appropriate to select the combination that would enable ease measurement (Chineke 2000). Thus with the aid of regression equation involving ear length and height at withers they appropriate values could be substituted to obtain estimate of rabbit body weight for the age bracket reported in this work. The result on the Dutch breed show that the trait combination involving ear length, heart girth and height at withers contributed 97.1% to body weight while combination of ear length and heart girth contributed 96.1% to body weight.

Table 6 - Simple Regression equations for the different variables on age for the three genotypes of rabbits

Body trait	Genotype	Intercept (a)	Regression coefficient (b)	Coefficient of R^2	SE
Body weight	NZW	81.245	0.743**	0.553	217.55
	Dutch	28.081	0.832**	0.693	204.166
	Chinchilla	-61.811	0.717**	0.514	343.44
Tail length	NZE	7.196	0.324**	0.105	1.937
	Dutch	6.300	0.533**	0.284	0.991
	Chinchilla	4.942	0.427**	0.183	1.495
Heart Girth	NZW	16.847	0.549**	0.301	1.139
	Dutch	16.865	0.423**	0.179	1.896
	Chinchilla	16.898	0.608**	0.369	1.492
Ear length	NZW	7.585	0.623**	0.389	0.560
	Dutch	7.664	0.772**	0.596	0.424
	Chinchilla	6.569	0.654**	0.427	0.845
Head of shoulder	NZW	6.912	0.420**	0.176	1.426
	Dutch	5.895	0.513**	0.263	1.048
	Chinchilla	5.853	0.422**	0.178	1.539
Height at withers	NZW	10.685	0.496**	0.246	0.654
	Dutch	11.199	0.659**	0.434	0.449
	Chinchilla	11.589	0.459**	0.210	0.894
Shoulder to tail drop	NZW	19.209	0.285**	0.081	2.358
	Dutch	19.299	0.614	0.376	1.381
	Chinchilla	18.029	0.377	0.142	2.889

SE= Standard Error. ** Significant at P<0.01

Table 7 - Stepwise regression equations for estimating body weight at 20 weeks of age, using different combinations of linear body traits.

Genotype	Step	Trait	Intercept	Regression coefficient	Partial R^2	Model R^2	SE
New Zealand	1	Ear length	-2966.339	0.958	0.919	0.917	92.79
	2	Ear length	-2113.960	1.755	0.947	0.945	75.69
		Height at Withers	-	-0.814	–	–	–
	3.	Ear length	-1400.271	1.778	0.951	0.949	72.98
		Height at Withers	–	-1.026	–	–	
		Tail length	–	0.202	–	–	–
Dutch	1	Ear length	-3990.328	0.983	0.966	0.966	67.501
	2	Ear length	-4079.025	1.056	0.969	0.968	65.743
		Height girth	–	-0.087			–
	3.	Ear length	-4650.660	0.909	0.971	0.970	63.474
		Heart girth	–	-0.134	–	–	
		Height at Withers	–	0.195	–	–	
Chinchilla	1.	Heart girth	-4009.619	0.967	0.935	0.933	125.97
	2.	Heart girth	-3718.471	1.216	0.940	0.938	121.53
		Height at Withers	–	-0.260	–	–	–

SE= Standard Error

CONCLUSSION

Result on the chinchilla proved that two combinations would best predict body weight at 20 weeks of age. Combination of heart girth and height at withers contributed 94.0% variability in body weight. This also corroborates the findings of Chineke (2000), who reported very high association for heart girth, height at wither and body weight.

The results indicate that with the various breeds of rabbits, producers can easily predict weight of rabbit breeds from any given value of the seven body measurements. Simple tape/meter rule can be used to take the measurement of the rabbits. Substituting of the values in the regression equation for 20 weeks of age reported in this work would give close value of the body weight of rabbits at this particular age. The results of this study indicate that weaner body weight of rabbits had positive and significant effect on production traits and linear body parameters. This suggested that heavy high weaner body weight would perform more creditably than those of opposite characteristics for selection and breeding/ production programmes.

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INFLUENCE OF DIETARY CALCIUM LEVELS ON BONE DEVELOPMENT IN BROILER BREEDER PULLETS UP TO 18 WEEKS OF AGE

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ABSTRACT: *The effects of three levels of dietary calcium on bone development in broiler breeder pullets up 18 weeks of age were investigated. A total of 640 one-day-old Ross broiler breeder pullets were used and were randomly assigned to four treatment groups, each having four replicates. The experimental design was split plot with four dietary treatments being the main plots and age as split plots. The four treatments were 1.0% Ca (0.45% Pi), 1.5% Ca (0.7% Pi), 2.0% Ca (0.9% Pi) and 1.0% Ca (0.45% Pi). The first 3 treatments were feed restricted according to Ross Breeders recommendations while the latter was ad libitum fed (served as control). Pullets were fed threeisocaloric and isonitrogenous diets: pre-starter (0 to 2 weeks); starter (2 to 4 weeks) and grower (4 to 18 weeks). At 6, 12 and 18 weeks of age, 5 pullets from each replicate were randomly selected and sacrificed by cervical dislocation and tibiae (left and right) and right humeri from each bird excised. Parameters studied were bone weight, bone length, bone width, bone ash, percent bone, true cortical area, bone strength and stress. These results showed that dietary Ca hadn't statistical (P>.05) influence on bone formation of broiler breeder pullets on restricted feeding, except bone strength. Ad libitum feeding of broiler breeder pullets resulted in a significant (P<.05) increase in bone dimensions and bone breaking strength. However, ad libitum birds had significantly (P<0.0001) lower bone stress values than restricted groups, indicating less mineralization. However, stress required to break bones from ad libitum birds was significantly (P<0.0001) lower than that required to break bones from restricted group. These results showed that dietary Ca level had no significant effect on bone formation in broiler breeder pullets on restricted feeding up to 18 weeks of age, except bone breaking strength.*

Keywords: Bone dimensions, bone strength, bone stress, calcium

ORIGINAL ARTICLE

INTRODUCTION

Broiler breeders are continuously selected for higher growth rate. Therefore, feed restriction during the growing and laying period is a common practice to prevent excess body weight (Ingram et al., 2001). Williams et al. (2000*a*) suggested that bone characteristics might be changing in modern fast growing broilers, with increases in cortical bone porosity and changes in composition that could affect mechanical properties of bone. Feed restriction in broiler breeders results in changes in the relative growth of different body components, but there is little information on the impact of this on bone structure and composition (McCormack et al. 2001).

In broiler breeders, Calcium (Ca) is associated with eggshell formation, but has many important functions in the body, of which the structuring of bones is the most important. About 99% of Ca is contained in the skeleton (Hurwitz et al., 1987). Several researchers (Shafey, 1993; Roberson et al., 2004) reported an increase in bone breaking strength (BS) because of increased levels of dietary Ca and phosphorus (P) in commercial growing and laying chickens as well as growing turkeys. Accordingly, higher bone ash percentages were recorded as the Ca level in over one year-old laying hen dietsincreased. Hulan et al. (1986) found that biological performance (body weight gain, final live weight and feed conversion) declines as the total Ca+avP and Ca: avP ratio increase in the diets.

Further research on higher Ca levels in broiler breeder pullets' diets is of utmost importance, as most of the studies regarding dietary Ca levels on bone development involved laying hens. Therefore, a study was undertaken to investigate the effects of dietary Ca levels and feed restriction on bone development of broiler breeders up to 18 weeks of age.

MATERIAL AND METHODS

Six hundred and forty one-day-old Ross broiler breeder pullets were obtained from a commercial hatchery and were randomly assigned to 4 treatment groups, each having 4 replicates. The 4 treatments were 1.0% Ca (0.45% Pi), 1.5% Ca (0.7% Pi), 2.0% Ca (0.9% Pi) and 1.0% Ca (0.45% Pi). The first 3 treatments were feed restricted according to Ross Breeders (2001) recommendations while the last treatment was *ad libitum* fed (served as control) throughout the rearing period (18 weeks). Feed restriction started at 2 weeks of age. Individual feeds were analysed for Ca and P to ensure accurate diet formulation. Water was provided *ad libitum* for all treatments. The initial weight of the birds was determined by weighing 5% of the birds prior to allocation to 4 dietary treatments.

Pullets were fed different diets during the 3 feeding phases of the experimental period namely, pre-starter (0 to 2 weeks); starter (2 to 4 weeks) and grower (4 to 18 weeks). The physical and nutrient compositions of diets are shown in Tables 1 and 2, respectively. The diets for each feeding phase were isocaloric and isonitrogenous. Calcium and feeding levels were the only differences during each specific phase. A diet with 1.5% Ca was obtained by mixing the 1.0% and 2.0% Ca diets.

Table 1 - Physical composition of experimental diets on air dry basis (%)

	Pre-starter diet		Starter		Grower	
	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca
Maize	58.62	58.15	58.15	58.37	67.12	64.82
Maize glutten	1.85	-	-	-	-	-
Wheat bran	6.50	12.00	12.00	5.45	12.0	9.30
Full fat soya	-	-	-	1.30	-	-
Soybean oil cake	17.85	17.85	17.85	18.95	6.70	11.60
Sunflower oil cake	8.00	8.00	8.00	8.00	10.0	6.40
Fishmeal	1.00	-	-	-	-	-
Calcium carbonate	1.30	1.45	1.45	3.00	1.70	2.95
Calcium monophosphate	1.27	1.31	1.31	3.37	1.47	4.08
Salt	0.17	0.23	0.23	0.24	0.23	0.26
Sodium bicarbonate	0.30	0.28	0.28	0.25	0.26	0.14
Choline liquid	0.03	0.021	0.02	0.03	0.052	0.05
Lysine	0.33	0.18	0.18	0.15	0.91	0.01
Threonine	0.33	-	-	-	-	-
Methionine	0.24	0.18	0.18	0.18	0.32	0.03
	0.35	0.35	0.35	0.35	0.35	0.35
Trace mineral / vitamin premix						

Day-old chicks were reared in 16 pens with 40 birds per pen and 4 pens (replicates) per treatment in a closed house with windows for ventilation. Each replicate was housed in floor pens, measuring 4 m^2 with wood shavings as litter material. The stocking density at 12 and 18 weeks of age was 0.11 and 0.13 per m^2, respectively as numbers decreased due to birds that were sacrificed for bone samples. Each pen was equipped with an electric brooder, 2 tube-type feeders and 2 automatic drinkers.

Pullets were reared at a time when day length was decreasing (May to July). Chicks received continuous light for the first 2 days of life and thereafter it was reduced to natural day length pattern of a decreasing and increasing photoperiod throughout the rearing period. Pullets were vaccinated in accordance with a vaccination programme obtained from a local parent stock company.

Feed consumption was measured by giving pre-weighed feed allocations to each replicate group throughout the week and then weighing back all of the unconsumed feed at the end of the week. Pen body weights (BW) were also recorded on weekly basis.

At 6, 12 and 18 weeks of age, 5 pullets were randomly selected from each replicate (i.e. 20 birds per treatment at each age) and killed by cervical dislocation and their carcasses stored in the refrigerator overnight and the bones removed the following day. The tibiae (left and right) and right humeri from each of the birds were excised and defleshed without boiling. The right tibiae and right humeri were then weighed and total length and bone shaft widths measured by means of a calliper with an accuracy of 0.001 cm (Zhang and Coon, 1997).

Table 2 - Nutrient composition of experimental diets on air dry basis (%)

	Pre-starter diet		Starter		Grower	
	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca	1.0% Ca	2.0% Ca
Moisture	11.19	11.31	11.31	10.93	11.20	10.96
ME (MJ/Kg)	12.10	11.80	11.80	11.60	12.10	11.70
Protein	20.26	17.99	17.99	17.99	14.00	14.32
Crude fat	3.01	3.05	3.05	3.05	3.26	3.12
Crude fibre	5.53	6.13	6.13	6.13	6.45	5.41
Calcium	0.99	1.01	1.01	2.00	1.10	2.01
Phosphorus	0.79	0.81	0.81	1.28	0.82	1.36
Available phosphorus	0.45	0.90	0.45	0.90	0.45	0.90
Arginine	1.25	1.15	1.15	1.16	0.88	0.90
Isoleucine	0.84	0.74	0.74	0.76	0.55	0.58
Methionine	0.59	0.49	0.48	0.48	0.30	0.29
TSAA[1]	0.95	0.81	0.81	0.81	0.58	0.57
Threonine	0.78	0.66	0.66	0.67	0.51	0.53
Tryptophan	0.23	0.21	0.21	0.21	0.16	0.16
TA[2] arginine	1.16	1.07	1.06	1.07	0.81	0.83
TA[2] isoleucine	0.76	0.67	0.67	0.69	0.49	0.55
TA[2] lysine	1.05	0.85	0.85	0.85	0.55	0.55
TA[2] methionine	0.56	0.45	0.45	0.45	0.27	0.51
TA[2] TSAA	0.87	0.73	0.73	0.73	0.51	0.46
TA[2] Threonine	0.69	0.58	0.58	0.59	0.45	0.46
TA[2] Tryptophan	0.21	0.19	0.19	0.19	0.14	0.15
Linoleic acid	1.59	1.68	1.68	1.65	1.82	1.72
Salt	0.21	0.23	0.23	0.25	0.24	0.27
Choline (mg/kg)	1410.68	1288.83	1288.83	1308.81	1311.38	1307.09
Sodium	0.18	0.18	0.18	0.18	0.18	0.16
Chlorine	0.24	0.22	0.66	0.66	0.22	0.22
Potassium	0.71	0.70	0.70	0.70	0.57	0.59

[1]Total sulphur amino acids, [2] Chemically determined

The tibiae and humeri were individually sealed in plastic bags to minimise moisture loss, and stored in a freezer at -18 ^{0}C for later analysis (Zhang and Coon, 1997). The bones were then removed for bone ash and BS determinations. The right tibiae and right humeri were used for BS while left tibiae were used for bone ash determination and histomorphometric analysis. Breaking strength (N) was determined according to procedures described by Fleming et al. (1998). Bone stress (N/mm^2), was calculated by dividing BS with true cortical area (mm^2). True cortical area (TCA) was calculated by multiplying cortical area with mean percent bone and divided by 100. Percent bone, which is the reciprocal of porosity, was determined from microscopic observations.

Left tibiae were dissected and a 5 mm ring from midshaft taken for histological processing. Two additional samples were taken, 20 mm on either side of the ring, and combined for ash measurements according to the procedures described by Fleming et al. (1998) and Williams et al. (2000*a*, 2000*b*). The bone cross-section taken for histology was fixed in 10% neutral buffered formalin, decalcified and processed for histomorphometric analysis according to the procedures described by (Fleming et al., 1998).

Bone data obtained at each sampling period were regressed on average Ca intake and/or levels per chicken during the particular period, namely from day-old to 6 weeks of age, from day-old to 12 weeks of age and for the entire period of day-old to 18 weeks of age. Calcium intakes were calculated from average feed intake values of the birds on a particular dietary Ca level. The Minitab Statistical Software package (Release 8.2) (Minitab Inc., 1991) was employed to analyse data sets.

In a second analysis of the data the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1996) was used to estimate differences between treatment means for the different levels of Ca intake within and between age periods. In this analysis, data were regarded as a split plot design with 4 dietary treatments being the main plots and age as split plots. The differences between treatment means were separated using the Tukey test.

RESULTS AND DISCUSION

Feed intake

Dietary Ca levels did not appear to influence feed intake. This is in line with the findings of Smith et al. (2003) who found that increasing dietary Ca level from 0.9 to 1.5% had no effect on feed consumption of broilers. Similar results were reported by Ahmad et al. (2003) in Bovans hens. On the other hand, Shafey and McDonald (1991) found that high dietary Ca (2.43 *vs.* 0.89%) reduced feed intake in broiler chicks reared up to 17 days of age. It seems that the influence of Ca levels on feed intake differs between *ad libitum* and feed restricted birds. In restricted birds, Ca levels within limits had no pronounced effect on feed intake.

Feed intake increased significantly ($P<0.05$) with age and the *ad libitum* group consumed significantly ($P<0.05$) more feed than restricted groups. The average feed intake of restricted birds (1.0% Ca diet) was 34.2% of that of the *ad libitum* fed birds (0-18 weeks). Yu et al. (1992a) reported average feed intake of restricted birds to be 37.2% of the full-fed (*ad libitum*) birds during rearing (4-18 weeks).

Calcium intake

The daily Ca intake of the birds significantly ($P<0.05$) increased with increasing dietary Ca level. The average daily Ca intake per bird during the rearing period for birds fed 1.0%, 1.5%, 2.0% Ca diets and *ad libitum*-fed birds was 0.66 g, 0.81 g, 1.0 g and 1.6 g, respectively. Accordingly, Yu et al. (1992b) reported daily average Ca intakes of 0.6 g for feed restricted and 1.67 g for *ad libitum* fed Indian River breeder hens from 4 to 18 weeks of age. In accordance with feed intake, Ca intake of the restricted birds in the present was significantly ($P<0.05$) lower than that of the *ad libitum* group.

Body weight

Dietary Ca levels had no statistical significant ($P<0.05$) influence on the growth rate of birds fed a restricted isonitrogenous and isocaloric diet. These results are consistent with Rosa et al. (2010). In disagreement with these results, Shafey and McDonald (1991) found that increased dietary levels of Ca alone or Ca (2.56%) and P (.49% available P) significantly ($P<0.01$) reduced body weight gain in broiler chickens. The results of the current study suggest that the NRC (1994) recommendations for broiler breeders (1.0% Ca) may be sufficient to support the required growth. As expected, the *ad libitum* group was significantly ($P<0.05$) heavier than restricted groups. In agreement with these results, Yu et al. (1992b) reported that restricted birds not only weighed less, but also had a significantly shorter length than *ad libitum*-fed birds, an indication of stunted growth.

Mortality

Overall mortality for the entire rearing period was 7.2% (46 birds), which is higher than the Ross Breeders standard mortality of 5.1% at 18 weeks of age. The mortality rate was 6.9%, 8.1%, 7.5% and 6.3% for birds on 1.0%, 1.5%, 2.0% Ca and *ad libitum* group, respectively. The high mortality observed in the restricted groups was mainly due to cannibalism as the birds were not debeaked. Atkinson et al. (1967) found that Ca levels *per se* had no significant influence on bird mortality during rearing.

Bone dimensions

Bone length

The mean values for tibia and humerus length are given in Table 3. It is evident that bone length increased non-significantly with increased dietary Ca intake. Restricted birds had shorter tibiae and humeri than *ad libitum* birds, an indication of stunted growth. Yu et al. (1992b) found that restricted birds had significantly shorter tibiae than *ad libitum* fed birds at 18 weeks of age.

A regression analysis of the tibia and humerus length data (Table 4) at all ages showed a highly significant response to Ca intake when data of the *ad libitum* group was included in the calculations. However, no significant response in tibia lengths due to Ca intake could be demonstrated for the restricted groups.

As shown in Table 3 the length of tibia increased significantly ($P<.0001$) with age up to 12 weeks of age while that of humerus increased with age throughout the rearing period. Tibia length increased by 46% and 13% between 6 and 12 weeks and 12 and 18 weeks, respectively. On the other hand,increases in humerus length of 37 and 10% were noted at 6 and 12 weeks and 12 and 18 weeks, respectively. These values show that bone development and growth in broiler breeder pullet is rapid during the first 12 weeks of age.

Bone width and weight

No significant influence of dietary Ca intake on bone width of restricted birds could be detected (Table 3).However, bone width showed a constant significant increase, as the birds got heavier because of age and *ad libitum* feeding. Accordingly Williams et al. (2000a) reported that heavier birds had longer and wider tibiotarsi.

The results of the regression analysis accordingly did not result in any response of bone width to increasing dietary Ca levels (Table 4). However, when the bones from the *ad libitum* group were included in the data set a significant response in bone dimensions to increasing Ca intake occurred (Table 4). The explanation for the significant responses in bone dimensions when data from the *ad libitum* group was included in the data set lies most probably in the larger bone mass that was due to *ad libitum* feeding.A significant ($P<0.0001$) Ca level x age interaction for bone

weight occurred, indicating that the influence of dietary Ca on bone weight varied during different periods. Therefore, the effect of dietary Ca levels on bone weight was compared statistically within each age and the effect of age within Ca levels (Table 3). Dietary Ca levels did not significantly (P>.05) influence bone weight of the restricted birds (Table 4). However, a tendency for tibia weight to decrease with increased dietary concentration was observed among the restricted group (Table 3). This result is consistent with Williams et al. (2000*b*) who reported a tendency for bone weight to decrease with increasing dietary Ca concentration.

Ad libitum feeding resulted in a heavier bone weight at all ages in agreement with McCormack et al. (2001). The study of Yu et al. (1992b) reported tibia weights of the restricted groups at 18 weeks to be 85% of the *ad libitum* group, whereas it was 88% of the *ad libitum* group in the present study.

Table 3 - Effect of calcium levels on bone dimensions of broiler breeder pullets during rearing

		Age (weeks)				Significance of effect (P)			
	Treatment	6	12	18	Means	Treatment	Age	Interaction	CV
Right tibia									
Length (mm)	1% Ca	71.84 ± 5.43	100.00 ± 22.65	118.47 ± 11.31	96.76^{b}	0.0064	0.0001	0.2692	70.71
	1.5% Ca	72.11 ± 7.01	109.54 ± 21.72	118.35 ± 8.09	98.50^{b}				
	2% Ca	71.71 ± 5.58	105.54 ± 6.46	118.24 ± 9.00	100.00^{ab}				
	1% Ca &*ad lib.*	92.63 ± 5.54	190.14 ± 26.09	134.52 ± 4.69	139.09^{a}				
	Means	77.07^{a}	122.39^{b}	126.30^{b}					
Width (mm)	1% Ca	4.90 ± 0.66	6.81 ± 0.38	7.10 ± 0.67	6.27^{b}	0.0001	0.0001	0.6769	9.40
	1.5% Ca	4.78 ± 0.48	6.62 ± 0.48	7.23 ± 0.67	6.21^{b}				
	2% Ca	4.98 ± 0.71	6.62 ± 0.62	7.30 ± 0.82	6.30^{b}				
	1% Ca &*ad lib.*	7.03 ± 0.55	8.92 ± 0.64	9.63 ± 0.82	8.53^{a}				
	Means	5.42^{a}	7.24^{b}	7.81^{c}					
Weight (g)	1% Ca	4.05 ± 1.03^{a}	11.08 ± 2.53^{a}	15.22 ± 4.29^{a}		0.0001	0.0001	0.0001	19.64
	1.5% Ca	4.35 ± 1.13^{a}	11.19 ± 3.12^{a}	14.57± 2.66^{a}					
	2% Ca	4.07 ± 1.12^{a}	11.00 ± 1.61^{a}	14.70 ± 2.78^{a}					
	1% Ca &*ad lib.*	10.82±1.50^{b}	20.48^{b}	26.65^{b}					
Right humerus									
Length (mm)	1% Ca	52.77 ± 3.80	70.44 ± 16	80.26 ± 5.60	67.82^{b}	0.0001	0.0001	0.0644	8.59
	1.5% Ca	53.38 ± 3.80	74.32 ± 4.1	80.60 ± 5.90	69.44^{a}				
	2% Ca	53.40 ± 4.80	73.60 ± 3.6	80.20 ± 3.80	69.07^{b}				
	1% Ca &*ad lib.*	65.80 ± 3.70	83.80 ± 4.4	86.30 ± 3.80	78.65^{a}				
	Means	56.33^{a}	75.46^{b}	81.86^{c}					
Width (mm)	1% Ca	4.40 ± 0.40	6.31 ± 0.40	6.61 ± 0.40	6.27^{b}	0.0001	0.0001	0.4970	9.27
	1.5% Ca	4.36 ± 0.40	6.23 ± 0.50	6.49 ± 0.60	6.21^{b}				
	2% Ca	4.45 ± 0.50	6.12 ± 0.30	6.59 ± 0.70	6.30^{b}				
	1% Ca &*ad lib.*	6.28 ± 0.40	7.91 ± 1.10	8.69 ± 0.60	8.53^{a}				
	Means	4.88^{a}	6.64^{b}	7.09^{c}					
Weight (g)	1% Ca	2.27 ± 0.77^{b}	5.95 ± 1.81^{a}	7.51 ± 2.86^{a}		0.0001	0.0001	0.0001	27.19
	1.5% Ca	2.50 ± 0.81^{b}	5.71 ± 1.82^{a}	6.53 ± 1.70^{a}					
	2% Ca	2.33 ± 0.64^{b}	5.56 ± 1.47^{a}	6.98 ± 1.83^{a}					
	1% Ca &*ad lib.*	5.69 ± 1.09^{c}	11.46 ± 2.29^{b}	15.09 ± 2.92^{a}					

*Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred. Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

The weight of the right tibia increased significantly (P<0.0001) for each 6 weeks increment up to 18 weeks. On the other hand, the weight of the right humerus increased significantly (P<0.0001) up to 12 weeks and thereafter flattened off. This finding is consistent with Fisher (1998) and Ross Breeders (2001) who stated that skeletal size in broiler breeder pullets is fixed at 12 weeks.

Bone mechanical properties

Breaking strength (BS) data for humeri and tibiae are shown in Table 5. A significant (P<0.0036) Ca level x age interaction for BS occurred. Although different Ca levels did not significantly (P>0.05) influence BS, birds fed 2.0% Ca diet tended to have greater BS than those fed 1.0% and 1.5% Ca diets. According to regression analyses (Table 6), however, tibia BS in the restricted groups responded significantly to increasing intakes of Ca at 6 weeks, as well as, at 12 weeks of age. The response in humerus BS was only significant for the data collected at 6 weeks of age. These findings are different from what was found for bone dimensions for pullets on restricted feeding. Only when data of the *ad libitum* group was included in the data sets that significant responses in bone characteristics were noted (Table 5). In agreement with Frost (1997) and Rath et al. (1999, 2000), tibia BS significantly (P<.0001) increased with age. From Table 5, dietary Ca levels did not have a significant effect on tibia stress in the restricted groups. This is in agreement with McCormack et al. (2001) who found no significant influence of Ca levels on the bone stress of 6 weeks old Cobb broiler breeder pullets on restricted feeding.

Stress required to break bones from *ad libitum* birds was significantly (P<0.0001) lower than that required to break bones from restricted group (Table 5). Crenshaw et al. (1981a) states that as bone mineralisation increases, maximum stress of the bone increases. According to these results the degree of bone mineralisation was greater for restricted groups than for the *ad libitum* group. Such finding is to be expected, as rapid growth does not allow enough time for the production of strong tissue, remodelling, and alignment of bone resulting in less mineralisation. These

results suggest that less mineralisation occurred in birds on *ad libitum* feeding, an observation that agrees with that of Nimmo et al. (1980).

Bone chemical composition

Different dietary Ca levels resulted in no significant differences in the ash, Ca and P content of the restricted groups (Table 7).The result on bone Ca is consistent with Hocking et al. (2002) and Smith et al. (2003) who reported no benefits of feeding growing turkeys and broilers diets containing Ca levels ranging from 0.6 to 1.5%. Although 2.0% Ca level in the current study did not significantly influence Ca content of bone of restricted birds, this level showed a slightly lower Ca value. It seems from the present results that too high levels of Ca (2.0% and more) could influence the Ca content of the bone detrimentally. The mechanism of suppression of bone calcification by high dietary levels of Ca remains unclear. The feeding of high levels of Ca during the rearing period of broiler breeder pullets could probably result in the body's mechanism for Ca mobilisation to malfunction and gear the body for high levels of excretion. The result on bone ash is consistent with Fard et al. (2010).

There was a significant (P<.0001) increase in bone ash and Ca with age (Table 7). However, bone P declined (P<.0007) with age. According to Table 8, these variables did not increase with age. Hocking et al. (2002) reported a decline in Ca, P and bone ash due to age in growing turkeys up to 13 weeks of age. Only the result on the P reported in the present study confirms the work of Hocking et al. (2002). The finding on bone ash in the current study is in agreement with Rath et al. (1999) who reported an increase in tibia bone ash of female broiler breeder chickens aged 7 and 72 weeks. The differences in results with regards to bone ash and Ca may be attributable to length of experiment and differences in animal species but not Ca levels, as levels of Ca used in the present study and that of Hocking et al. (2002) were similar. The decline in bone ash values from 33.7 to 30% between 6 and 12 weeks of age could be associated with the birds increased demand for nutrients, notably Ca due to rapid growth rates. Accordingly, skeletal size is fixed at 12 weeks of age (Fisher, 1998; Ross Breeders, 2001). This pattern in bone development probably explains the high bone ash content at 18 weeks of age (Table 7).

Bone ash values obtained at 6 weeks age for the restricted groups in this study are consistent with the results of McCormack et al. (2001) who reported ash value of 33.63% after feeding Cobb broiler breeder pullets' diets containing 0.9 g Ca and 5.8 g total P during a 6-week period. In the current study, the average bone ash value for the birds on restricted feeding was 33.97% at 6 weeks. However, McCormack et al. (2001)) found a higher bone ash value (40.65%) for *ad libitum* group compared to 33.0% in the current study. The differences in the results relating to bone ash values for the *ad libitum* group could be attributable to differences in housing (*e.g.*, open-sided *vs.* climate controlled systems) and strain of birds (Ross *vs.* Cobb).

According to Ruff and Hughes (1985), bone ash content is correlated with its BS. However, this does not appear to be supported by the results of the current study. In this study, Ca and P contents did not increase significantly with increasing Ca level (Tables 7 and 8) while BS did (Table 6). Bone stress values in this study suggested that less mineralisation occurred in birds on *ad libitum* feeding.

True cortical area and percent bone

The means for true cortical area (TCA) and percent bone are presented in Table 7. A significant (P<.05) Ca level x age interaction for TCA and percent bone occurred, indicating that not all bones responded similarly at each age period or Ca level. A regression analysis of the two parameters at all ages (Table 8) showed a significant (P<.001) response to Ca intake when data of the *ad lib* group was included in the calculations. However, dietary Ca did not have significant (P>.05) influence on TCA and percent bone of the restricted birds (Tables 8).

Percent bone and TCA of restricted birds was not significantly (P>.05) different at 12 and 18 weeks, perhaps indicating that the bone is fully developed at 12 weeks (Table 7), thus confirming the theory that bone in broiler breeder pullets is fixed at 12 weeks of age.

Ad libitum birds had significantly (P<.05) higher TCA values than restricted birds (Table 7) probably due to faster growth rate. At 6 weeks, percent bone for *ad libitum* group was lower than that of restricted treatments but appeared to be similar to that of restricted birds at 12 and 18 weeks of age. The lower percent bone of the *ad libitum*birds may be attributable to rapid growth rate due to higher feed consumption rates. This probably indicates that the rate of mineralisation could not keep pace with rapid growth rate of the *ad libitum* group.

The TCA and percent bone were significantly (P<.0001) influenced by age (Table 7). The two parameters increased significantly from 6 to 12 weeks and then significantly flattened off. Between 6 and 12 weeks TCA and percent bone increased by 59.3% and 9.9%, respectively.

CONCLUSION

These results showed that dietary Ca level had no significant effect on bone formation in broiler breeder pullets on restricted feeding up to 18 weeks of age, except bone breaking strength. The results found for breaking strength were, however, not supported by stress required to break bones, percent bone ash, Ca and P contents as well as percent bone.

Table 4 - Multiple regression analysis of bone dimensions on calcium intake of pullets during rearing

		Data from restricted groups only			Data of *ad lib* group included		
Age	**Variable**	**Equations**	**Adj-R^2**	**P value**	**Equation**	**P value**	**Adj-R^2**
6 weeks	Tibia length	Tiblength6 = 72.50 - 0.83 Caint6		0.893	Tiblength6 = 52.2 + 26.2 Cain6	0.000	0.753
	Humerus length	Humlength6 = 52.10 + 1.33 Caint6	0.000	0.755	Humlength6 = 41.0 + 16.2 Caint6	0.000	0.583
	Tibia width	Tibwidth6 = 4.54 + 0.446 Caint6		0.486	Tibwidth6 = 2.79 + 2.77 Caint6	0.000	0.645
	Humerus width	Humwidth6 = 4.30 + 0.152 Caint6	0.000	0.732	Humwidth6= 2.62 + 2.38 Caint6	0.000	0.711
	Tibia weight, g	Tibweight6 = 4.23 - 0.01 Caint6		0.995	Tibweight6 = 1.89 + 8.14 Caint6	0.000	0.699
	Humerus weight	Humweight6 = 2.45 – 0.103 Caint6	0.000	0.892	Humweight6 = -0.789 + 4.21 Caint6	0.000	0.655
12 weeks	Tibia length, mm	Tiblength12 = 107.00 - 0.39 Caint12		0.956	Tiblength12 = 92.3 + 15.2 Caint12	0.000	0.639
	Humerus length	Humlength12 = 68.30 + 4.53 Caint12	0.000	0.355	Humlength12 = 65.5 + 7.41 Caint12	0.000	0.229
	Tibia width	Tibwidth12 = 6.96 - 0.278 Caint12		0.272	Tinwidth12 = 5.39 + 1.37 Caint12	0.000	0.639
	Humerus width	Humwidth12 = 6.52 – 0.297 Caint12	0.000	0.148	Humwidth12 = 5.26 + 1.02 Caint12	0.000	0.449
	Tibia weight	Tibweight12 = 10.80 + 0.30 Caint12		0.769	Tibweight12 = 5.40 + 5.94 Caint12	0.000	0.699
	Humerus weight	Humweight12 = 6.34 – 0.598 Caint12	0.000	0.482	Humweight12 = 2.41 + 3.51 Caint12	0.000	0.542
18 weeks	Tibia length	Tiblength18 = 119.00 - 0.24 Caint18		0.939	Tiblength18 = 107 + 9.91 Caint18	0.000	0.230
	Humerus length	Humlength18 = 80.50 - 0.07 Caint18	0.000	0.966	Humlength18 = 76.0 + 3.67 Caint18	0.000	0.127
	Tibia width	Tibwidth18 = 6.93 + 0.203 Caint18		0.389	Tibwidth18 = 5.30 + 1.58 Caint18	0.000	0.436
	Humerus width	Humwidth = 6.57 – 0.009 Caint18	0.000	0.962	Humwidth18 = 5.01 + 1.31 Caint18	0.000	0.424
	Tibia weight	Tibweight18 = 15.70 - 0.61 Caint18		0.583	Tibweight18 = 6.67 + 7.00 Caint18	0.000	0.376
	Humerus weight	Humweight18 = 7.68 – 0.502 Caint18	0.000	0.487	Humweight8 = 1.39 + 4.80 Caint18	0.000	0.368

Tib = tibia; Hum = humerus; Caint = calcium intake

Table 5 - Effect of calcium levels on bone mechanical properties of broiler breeder pullets during rearing

	Treatment	Age				Significance of effect (P)			
		6	12	18	Means	Treatment	Age	Interaction	CV
Right tibia									
Bone strength (N)	1% Ca	97.65 ± 22.23^{c}	213.90 ± 22.23^{b}	269.78 ± 22.23^{a}		0.0001	0.0001	0.0030	17.43
	1.5% Ca	93.30 ± 22.23^{b}	221.00 ± 22.23^{a}	249.28 ± 22.23^{a}					
	2% Ca	108.55 ± 22.23^{b}	253.50 ± 22.23^{a}	268.15 ± 22.23^{a}					
	1% Ca & *ad lib*	298.30±22.23^{c}	387.50±22.23^{b}	599.5± 22.23^{a}					
Bone stress (N/mm^2)	1% Ca	23.52 ± 1.51	19.0 ± 1.51	23.09 ± 1.51	21.87^{b}	0.0001	0.0003	0.1951	14.80
	1.5% Ca	24.86 ± 1.51	20.25 ± 1.51	26.84 ± 1.51	23.98^{b}				
	2% Ca	21.16 ±1.51	19.23 ± 1.51	26.89 ± 1.51	22.43^{b}				
	1% Ca & *ad lib*	11.23 ± 1.51	12.89 ± 1.51	15.22 ± 1.51	13.11^{a}				
	Means	20.19^{ab}	17.84^{a}	23.01^{b}					
Right humerus									
Bone strength (N)	1% Ca	93.65 ± 29.38^{b}	236.00 ± 29.38^{a}	273.85 ± 29.38^{a}		0.0001	0.0001	0.0036	20.70
	1.5% Ca	90.20 ± 29.38^{b}	258.50 ± 29.38^{a}	262.63 ± 29.38^{a}					
	2% Ca	120.70 ± 29.38^{b}	276.50 ± 29.38^{a}	294.43 ± 29.38^{a}					
	1% Ca & *ad lib*	294.60 ± 29.38^{c}	501.00 ± 29.38^{b}	704.38 ± 29.38^{a}					

Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred. Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

Table 6 - Multiple regression analysis of bone mechanical properties data on calcium intake of boiler breeder pullets during rearing

		Data from restricted groups only			*Ad libitum* group data included		
Sampling age	**Variables**	**Equations**	**P value**	**Adj-R^2**	**Equations**	**P value**	**Adj-R^2**
6 weeks	Tibia breaking strength	RTib6 = 53.4 + 57.8 Caint6	0.029		RTib6 = -98.70 + 261 Caint6	0.000	0.793
	Humerus breaking strength	RHum6 = 21.1 + 104 Caint6	0.021		Rhum6 = -93.7 + 257 Caint6	0.000	0.740
	Bone stress	Stress6 = 27.2 – 6.6 Caint6	0.545		Stress6 = 30.5 – 10.9 Caint6	0.006	0.000
12 weeks	Tibia breaking strength	Rtib12 = 165.0 + 64.7 Caint12	0.008		RTib12 = 124 + 107 Caint12	0.000	0.608
	Humerus breaking strength	RHum12 = 201.0 + 55.8 Caint12	0.055		Rhum12 = 102 + 160 Caint12	0.000	0.730
	Bone stress	Stress12 = 19.4 + 0.13 Caint12	0.960		Stress12 = 23.2 – 3.92 Caint12	0.001	0.000
18 weeks	Tibia breaking strength	Rtib18 = 252.0 + 4.4 Caint18	0.855		RTib18 = 4.3 + 213 Caint 18	0.000	0.366
	Tibia breaking strength	Rhum18 = 251.0 + 21.2 Caint18	0.549		Rhum18 = -36.8 + 264 Caint18	0.000	0.415
	Bone stress	Stress18 = 20.5 + 3.79 Caint18	0.232		Stress18 = 31.3 – 5.31 Caint18	0.061	0.000

Rtib = right tibia; RH = right humerus; Caint = calcium intake

Table 7 - Bone chemical composition of broiler breeder pullets reared on different calcium levels in feed

	Treatment	Age (weeks)				Significance of effect (P)			
		6	12	18	Means	Treatment	Age	Interaction	CV
Left tibia									
Ash content (%)	1.0% Ca	34.43 ± 1.70	29.31 ± 1.70	58.40 ± 1.70	40.71± 0.91[a]	0.8606	0.0001	0.4952	8.32
	1.5% Ca	33.91 ± 1.70	29.53 ± 1.70	60.93 ± 1.70	41.46 ± 0.91[a]				
	2.0% Ca	33.57 ± 1.70	32.24 ± 1.70	56.37 ± 1.70	40.73 ± 0.91[a]				
	1.0% Ca & *ad lib*	33.00 ± 1.70	29.07 ± 1.70	58.76 ± 1.70	40.27 ± 0.91[a]				
	Means	33.73 ± 0.85[a]	30.04 ± 0.85[b]	58.62 ± 0.84[c]					
Calcium, %	1.0% Ca	26.77 ± 3.62	29.19 ± 3.62	35.28 ± 3.62	30.41 ± 0.79[a]	0.8228	0.05	0.9199	24.62
	1.5% Ca	27.57 ± 3.62	30.74 ± 3.62	32.82 ± 3.62	30.37 ± 0.79[a]				
	2.0% Ca	26.70 ± 3.62	29.00 ± 3.62	29.21 ± 3.62	28.30 ± 0.79[a]				
	1.0% Ca & *ad lib*	24.13 ± 3.62	26.75 ± 3.62	34.64 ± 3.62	28.51 ± 0.79[a]				
	Means	26.29 ± 1.81[a]	28.92 ± 1.81[a]	33.00 ± 1.81[b]					
Phosphorus, %	1.0% Ca	17.71 ± 0.87	16.63 ± 0.87	15.95 ± 0.87	16.76 ± 47[a]	0.3785	0.0007	0.7347	10.64
	1.5% Ca	18.26 ± 0.87	17.03 ± 0.87	14.86 ± 0.87	16.72 ± 47[a]				
	2.0% Ca	17.05 ± 0.87	17.67 ± 0.87	14.24 ± 0.87	16.32 ± 47[a]				
	1.0% Ca & *ad lib*	16.95 ± 0.87	15.68 ± 0.87	14.28 ± 0.87	15.64 ± 47[a]				
	Means	17.49 ± 0.43[a]	16.75 ± 0.43[a]	14.83 ± 0.43[b]					
TCA[1] (mm^2)	1.0% Ca	6.08 ± 0.71[b]	12.25 ± 0.71[a]	13.35 ± 0.71[a]		0.0001	0.0001	0.0458	10.66
	1.5% Ca	6.44 ± 0.71[b]	12.65 ± 0.71[a]	13.15 ± 0.71[a]					
	2.0% Ca	6.72 ± 0.71[b]	13.52 ± 0.71[a]	13.40 ± 0.71[a]					
	1.0% Ca & *ad lib*	17.70 ± 0.71[b]	20.25 ± 0.71[b]	24.60 ± 0.71[a]					
Percent bone	1.0% Ca	83.52 ± 0.81[b]	89.86 ± 0.81[a]	92.46 ± 0.81[a]		0.0017	0.0001	0.0047	1.85
	1.5% Ca	83.13 ± 0.81[b]	90.46 ± 0.81[a]	91.10 ± 0.81[a]					
	2.0% Ca	82.50 ± 0.81[b]	91.43 ± 0.81[a]	91.93 ± 0.81[a]					
	1.0% Ca & *ad lib*	77.08 ± 0.81[b]	90.32 ± 0.81[a]	90.79 ± 0.81[a]					

[1]TCA – true cortical area (cortical area multiplied by mean % bone divided by 100). Means with the same letter within a column (treatment) or row (age) are not significantly different for the same variable, where no significant (P>0.05) interaction occurred. Means with the same letter within a row (age) are not significantly different for the same variable, where a significant (P<0.05) interaction occurred.

Table 8 - Multiple regression analysis of bone chemical composition on calcium intake of pullets during rearing

		Data from restricted groups only			Data of *ad lib* group included		
Age	**Variable**	**Equations**	**P value**	**Adj-R^2**	**Equation**	**P value**	**Adj-R^2**
6 weeks	Ash, %	%Ash6 = 26.20 + 10.1 Cain6	0.090	0.032	%Ash6 = 35.4 – 2.2 Caint6	0.312	0.000
	Percent bone	%bone6 = 86.6 – 3.25 Cain6	0.204	0.000	%bone = 89.1 – 7.95 Caint6	0.000	0.780
	True cortical area, mm^2	TCA6 = 5.13 + 1.57 Cain6	0.360	0.000	TCA6 = -4.53 + 14.5 Caint6	0.000	0.873
12 weeks	Ash, %	%Ash12 = 27.7 + 1.77 Cain12	0.228	0.008	%Ash12 = 29.5 – 0.117 Caint12	0.823	0.000
	Percent bone	%bone12 = 88.1+ 2.48 Cain12	0.609	0.081	%bone = 90.3 + 0.133 Caint12	0.854	0.000
	True cortical area, mm^2	TCA12 = 10.7 + 2.00 Cain12	0.052	0.140	TCA12 = 8.03 + 4.90 Caint12	0.000	0.854
18 weeks	Ash, %	%Ash18 = 62.4 - 1.83 Cain18	0.326	0.000	%Ash18 = 66.3 – 5.14 Caint18	0.000	0.000
	Percent bone, %	%bone18 = 92.5- 0.45 Cain18	0.305	0.000	%bone318 = 92.9 – 0.837 Caint18	0.103	0.120
	True cortical area, mm^2	TCA18 = 13.3 + 0.013 Cain 18	0.988	0.000	TCA18 = 4.96 + 7.02 Caint18	0.001	0.516

TCA = true cortical area

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MICRONUCLEI PROFILE: AN INDEX OF CHROMOSOMAL ABERRATIONS IN FRESHWATER FISHES (*SYNODONTIS CLARIAS AND TILAPIA NILOTICA*)

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ABSTRACT: *Incidence of chromosomal aberrations in Synodontis clarias and Tilapia nilotica (Linnaeus 1757) were measured using the conventional micronucleus assay in fish erythrocytes. The species showed varying degree of micronuclei frequencies in their respective genomes of sampled gill and kidney blood. Cytological examinations showed bi-nucleated cells, deformed nuclei including the main aberrations, micronucleus formations in various genomes of the fish from different locations considered in this study. Comparison of the micronucleus rates in peripheral and kidney blood of the two species revealed no statistical difference (P> 0.05). On species occurrence of the measured chromosomal aberrations, averages of micronucleus frequencies recorded in Synodontis clarias showed visible variation and to be 2.2 folds higher than the values obtained in the corresponding Tilapia sp. but there was no statistical difference (P>0.01) among the two breeds. The work recommends that micronuclei tests in fish erythrocytes be carried out at various times, thus making it possible to follow-up the changing micronuclei frequencies and concludes that gills and kidney erythrocytes can be used in studies concerning chromosomal aberrations since the sampling of the peripheral blood is appropriate as it allows collecting several samples from the same individuals, without having to sacrifice it.*

ORIGINAL ARTICLE

Keywords: *Micronucleus assay, chromosomal aberrations, Synodontis clarias, Tilapia nilotica, Anambra River*

INTRODUCTION

Chromosomal and cytogenetic studies on fish have received considerable attention in recent years (Okonkwo and Obiakor 2010; Galetti et al 2000; Ozouf-Costaz and Foresti 1992). Fish chromosome data have great importance in studies concerning evolution, systematics, aquaculture and mutagenesis (Amemiya 1986; Al-Sabti 1991).

The erythrocyte micronucleus bioassay has been used with different fish species to monitor aquatic pollutants displaying mutagenic features (De Flora et al 1993). Kligerman (1982) demonstrated that fishes inhabiting polluted waters have greater frequencies of micronuclei compared to those raised in clean pond. The micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. These structures are easy to visualize in erythrocytes and are therefore often used as a measure of chromosomal aberrations (Rabello-Gay 1991; Hartwell et al 2000).

Odo et al (2009) reported that *Tilapia nilotica* and *Synodontis clarias* are the most preponderant species of fish found in the Anambra River, and constitute the main diet for over one million rural dwellers living along the river bank.

Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation, the incidence of micronuclei serves as an index of these types of damage (Fagr et al 2008). Because counting of micronuclei is much faster and less technically demanding, the micronucleus assay has been widely used

to screen for chemicals that cause these types of damage (Fagr et al 2008) and the damage resulting from it. The failure of two sister chromatids to separate during mitotic anaphase generates reciprocal trisomic and monosomic daughter cells. Mistakes such as a lagging chromatid not pulled to either spindle pole at mitotic anaphase, result in a chromosome loss that produces one monosomic daughter cell. These result in classic deviation from the normal chromosomal diploidy (Hartwell et al 2000).

Changes in chromosome number evidenced by micronucleus formations may affect gene activity or gene transmission by altering the position, order, or number of certain genes in a cell. Such changes often, but not always, lead to a genetic imbalance that is harmful to the organism or its progeny (Hartwell et al 2000). Going by the same author, if the chromosomal inversion (half-circle rotation of a chromosomal region) is paracentric and a crossover occurs within the inversion loop, the recombinant chromatids will be unbalanced not only in gene dosage, but also in centromere number. One crossover products will be the main chromosomal aberration, an acentric fragment lacking a centromere; while the reciprocal crossover product will be a dicentric chromatid with two centromeres. Because the acentric fragment without a centromere cannot attach to the spindle apparatus during the first meiotic division in reproduction, the cell cannot package it into either of the daughter nuclei; as a result, this chromosome is lost and will not be included in a gamete.

In fish, the kidney is responsible for erythropoiesis as well as filtration. Upon fish exposure to toxins, defective erythrocytes undergo passage from the kidney into the peripheral blood, from where they are removed by the hemocatheresis organs (Palhares and Grisolia 2002). One of the hypotheses of this study was that the examination of kidney erythrocytes would provide more sensitive detection of micronuclei frequencies than peripheral blood erythrocytes under natural conditions. Chromosomal aberration studies with preponderant native fish species represent an important effort in delineating the extent of a particular chromosome damage and change such as micronuclei formations and likely agents inducing the visible aberration in the fish genome. The study was carried out to evaluate chromosomal aberration of micronucleus formation in genotypes of the two preponderant fish species in Anambra River under natural conditions using micronucleus test.

MATERIAL AND METHODS

Study Area

Anambra State in Nigeria lies between latitude 5^0 40'N and 6^0 45'N and longitude 6^0 35'E and 7^0 21'E. The climate is tropical with average annual rainfall of 200mm and mean temperature of 27^0C (Anyanwu 2006). The study area, which is Anambra River spatially lies between latitude 6^0 00'N and 6^0 30'N and longitude 6^0 45'E and 7^0 15'E. The river on the other hand is located in the South Central region of Nigeria, just close to the East of the Niger River into which it empties (Awachie and Hare 1977). Anambra River is approximately 207.4 km to 210 km in area (Odo 2004; Shahin 2002), rising from the Ankpa hills (ca. 305-610m above sea level) and discharging into River Niger at Onitsha (Odo 2004). The entire river basin drains an area of approximately 14010km^2 (Awachie and Hare 1977).

Sampling Stations

The sampling stations were established to cover possibly the whole area along the river course based on an earlier field reconnaissance tour. The locations (L_x) of the various sampling stations are;

L_A = Enugu Otu (Station A): Rice production and farming site.

L_B = Ezi Aguleri (Station B): Farming, Fishing and effluent discharge.

L_C = Otuocha (Station C): Rice production, marketing activities and waste disposal.

L_D = Otu Nsugbe (Station D): Wastewater effluent discharge, farming and marketing activities.

L_E = Onono (Station E): Sand mining and excavation, agricultural activities, fishing and sewage disposal. The location is close to Onitsha metropolis and mouth of Oyi River, the repository of industrial/domestic effluents.

Sample Collection

Live *Synodontis clarias* and *Tilapia nilotica* (Linnaeus 1757) of fairly similar live weight were collected from Anambra River at the five locations/stations using set nets, long-lines and traps. The relative distance between each station was approximately 12 km and all the sample collections were made during the morning hours at the peak of rainy season in the month of July, 2009.

Micronucleus Test

Blood samples were collected from caught fish. The peripheral blood smears were obtained through the gills and kidney blood by means of a medial-kidney imprint following dissection as described by Fagr et al (2008) and Palhares and Grisolia (2002). The slides were then, air-dried for 24h, fixed in methanol for 10min, followed by 10% Giemsa (v/v) staining. 2000 erythrocytes of each fish were examined, from both peripheral blood and the kidney. To determine micronuclei in erythrocytes, the slides were examined using oil-immersion (x 1000). For the scoring of micronuclei, the following criteria were adopted from Fenech et al (2003); the diameter of the micronucleus (MN) should be less than one-third of the main nucleus; MN should be separated from or marginally overlap with main nucleus as long as there is clears identification of the nuclear boundary; and MN should have similar staining as the main nucleus.

Data Analysis

Statistical analysis was performed using Student's *t* test.

RESULTS

Results reveal that the two fish species represent various degrees of sensitivity in monitoring genetic damage (especially clastogenic effect). This is indicated by variations in averages of the micronucleated cells among species at various locations/ stations. The obtained results are summarized in Table 1 and Figure 1 - 3. The chromosomal aberrations represented by the formation of micronucleus showed marked increase in the following level of occurrences; A<B<D<C<E. Location/station E was observed to possess fish with higher level of micronucleus frequencies.

Generally, the spontaneous micronuclei frequencies observed in kidney and in peripheral blood erythrocytes were statistically not different (P>0.05, Table 1), indicating no difference between kidney and gill in both *Synodontis clarias* and *Tilapia nilotica* (P=1.988, Table 1). Micronuclei formation in *Synodontis clarias* was visibly found to be higher than the levels detected in *Tilapia nilotica* as shown in Table 1, the averages of micronucleated erythrocytes formed in kidneys and gills of different genomes of fish at different locations. The results revealed that the micronucleus percentages were proven to be 2.2 folds higher in *Synodontis clarias* than in *Tilapia nilotica*, although the observed differences were not statistically significant (P=1.861) at 1% level of significance. Figure 1 shows micronucleated cells in different fish species caught from the five locations (stations) studied. Cytological evaluation (Baker et al 1998) revealed binucleated and deformed nucleus including the main type of chromosomal aberrations (micronucleus) observed. These are shown in Figure 2 and 3.

Table 1 - Mean (± SE) of micronucleus frequencies (MN/1000 erythrocytes) examined in gill and kidney blood of fish sourced from different locations in Anambra River.

	Species (Erythrocytes)			
Station	*T. nilotica* (Gill)	*T. nilotica* (Kidney)	*S. clarias* (Gill)	*S. clarias* (Kidney)
A	0.4±0.3 [ab]	0.2±0.3 [ab]	1.4±0.8 [ab]	0.3±0.4 [ab]
B	0.3±0.4 [ab]	0.2±0.3 [ab]	2.7±1.1 [ab]	1.8±1.8 [ab]
C	3.0±2.2 [ab]	1.6±0.8 [ab]	5.3±1.9 [ab]	2.1±0.4 [ab]
D	2.3±1.5 [ab]	2.1±1.0 [ab]	4.9±2.9 [ab]	1.6±0.8 [ab]
E	4.8±2.1 [ab]	2.1±1.2 [ab]	6.4±3.1 [ab]	4.6±1.7 [ab]

[a] significantly not higher (P= 1.861) at 1% level of significance, comparing the two specie.
[b] significantly not higher (P= 1.988) at 5% level of significance, comparing gill and kidney.

DISCUSION

Micronucleus bioassay offers several types of unique information as a bioindicator for chromosomal aberrations not available from other methods: (1) the integrated effect of a variety of environmental stresses on the health of an organism and the population, community, and ecosystem; (2) early warning of potential harm to human health based on the responses of wildlife to pollution; and (3) the effectiveness of remediation efforts in decontaminating waterways (Villela et al 2006). In fish, the micronucleus test is usually based on erythrocytes, but liver and gill tissues have been used (Al-Sabti and Metcalfe 1995).

Palhares and Grisolia (2002) compared between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish, following mitomycin C treatment detecting no significant difference between the frequencies of the micronuclei. Similarly, Manna and Sadhukan (1986) maintained that there was no statistically significant difference between the frequency of micronuclei in gill and kidney cells after irradiation in the two tissues. While they included various types of cells, our study was focused on the erythrocytes. A hypothesis to explain the fact that we did not detect any difference between kidney and peripheral blood micronuclei counts may be that circulating peripheral erythrocytes also undergo mitosis (Palhares and Grisolia 2002). However, if the kidney is the main hemopoietic tissue in fish, and if micronuclei are formed during cell proliferation (Palhares and Grisolia 2002; Hartwell et al 2000), more micronucleated erythrocytes should be expected in the kidney than in the gill. Alternatively, as reported by Palhares and Grisolia (2002), we may have sampled peripheral blood during kidney imprinting and practically, the cephalic kidney is a frequently chosen organ for cytogenetic aberration studies in fish. There was no significant difference (P> 0.05) between the frequencies of micronuclei obtained in *Synodontis clarias* and *Tilapia nilotica*. Fagr et al (2008) observed the African walking catfish, *Clarias gariepinus* from freshwaters of Egypt to have higher incidence of the chromosomal aberrations of micronuclei in its genome than the three tilapia species employed in the study, maintaining the species to be highly tolerant of that particular genetic damage without triggering the genetically programmed event that allows cells to commit suicide (Fagr et al 2008). Hence, the statistical results of no difference between the species of catfish and tilapia in our own study might be as a result of seasonal effect, physiological

variations and responses to the local agents inducing the chromosomal damage, which as advocated by Kligerman (1982), the micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. Sampling was carried out at peak of the tropical rainfall in July.

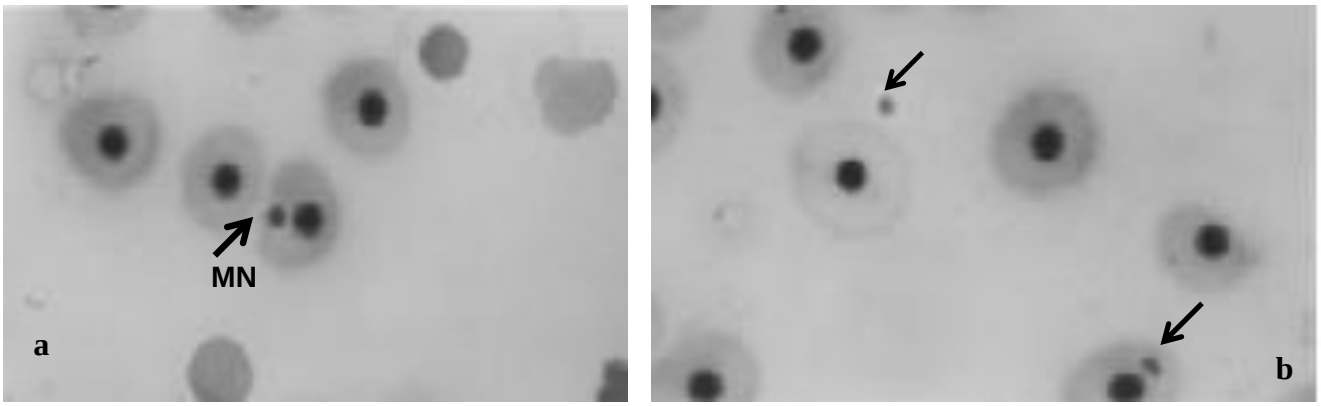

Fig. 1- Photomicrographs showing micronucleated erythrocyte (MN) from *Synodontis clarias* and *Tilapia nilotica* caught from location or station C (a) and E (b), respectively.

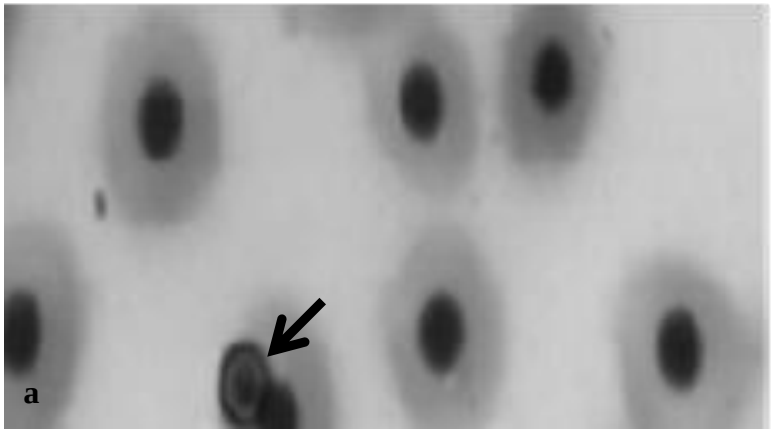

Fig. 2 - Photomicrograph showing binucleated erythrocyte (a) in *Tilapia nilotica* from location or station E.

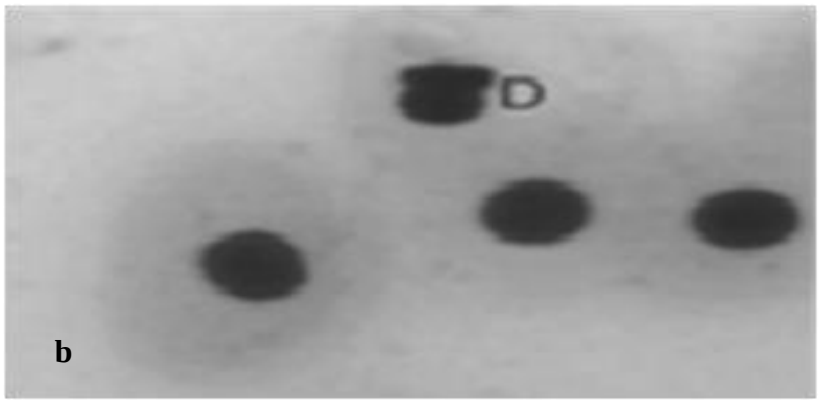

Fig. 3 - Photomicrograph showing Deformed Nucleus (D, b) in kidney blood of *Tilapia nilotica* sourced from location or station C.

At anaphase of meiosis 1, opposing spindle forces pull the dicentric chromatid toward both spindle poles at the same time with such strength that the dicentric chromatid breaks at random positions along the chromosome. These broken chrmomosome fragments (micronuclei) are deleted for many of their genes. This loss of the acentric fragments, together with breakage of the dicentric chromatid results in genetically unbalanced gametes, which at fertilization are lethal to the zygote's development. Consequently, no recombinant progeny resulting from such aberration survive except non recombinant progeny (Hartwell et al 2000). This observation lends substance to the abstract exposition of the foregoing drastic decline in fish diversity reported at Nsugbe, Ogurugu and Otuocha axes of

the Anambra River by Odo et al (2009), which had earlier been mentioned by Ndakide (1988) in the entire river, attributing it to recent anthropogenic influences.

Karyotypes generally remain constant within a species, not because changes in chromosome number occur infrequently (they are, in fact, quite common), but because the genetic instabilities and imbalances produced by such chromosome changes usually place individual cells or organisms and their progeny at a selective disadvantage (Hartwell et al., 2000). *Synodontis clarias* from the current work shows relatively high chromosomal anomalies than *Tilapia nilotica* and might hypothetically possess stable karyotype, contrasting observations made in a similar species, *Clarias gariepinus* (Burchell 1822) by Okonkwo and Obiakor (2010). The authors documented karyotypic polymorphism in *Clarias gariepinus* (Burchell 1822) from the Anambra River. The observed polymorphism in that species might be that the species genome well tolerates such type of cytogenetic damage (micronucleus) without apoptosis and as such fewer aberrations will be expected on the chromosomes of *Clarias gariepinus* than the former. It is suggested that karyological analysis of *Synodontis clarias* be carried out in Anambra River to establish its karyotypic forms and stability, including the chromosomal aberration status of *Clarias gariepinus* to portray the relationships with their karyotypes.

CONCLUSION

The results demonstrated that different fish species can respond in completely different ways to a given genotoxic agent. Depending on the toxic agent and on the species, the behavior of micronuclei rates may exhibit significant variations, probably related to the chemical kinetics of the toxins and to the speed of the hemopoietic cycle (Kligerman 1982). It is recommended that micronuclei tests in fish erythrocytes be carried out at various times, thus making it possible to follow-up the changing micronuclei frequencies. It is concluded from the study that gills and kidney erythrocytes can be used in studies concerning chromosomal aberrations. The sampling of the peripheral blood is appropriate since it allows collecting several samples from the same individuals, without having to sacrifice it (Lyne et al 1992).

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