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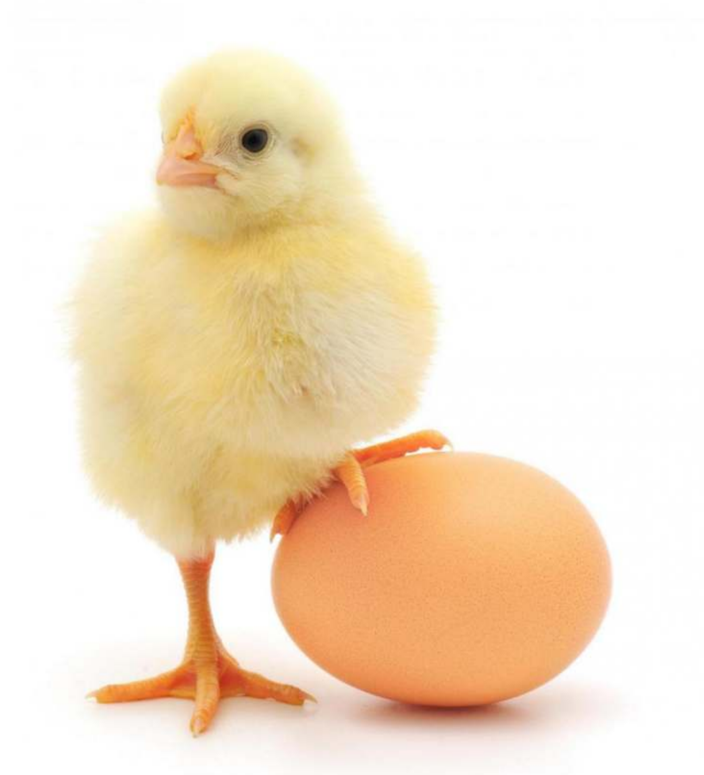

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Research Article
Effect of Diet Energy Level on the Histological Characteristics of Testes of Indigenous Barred Cock in the Western Highlands of Cameroon

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Abstract
Background and Objective: This study was conducted to determine the effect of diet energy level on the histological traits of testes. **Materials and Methods:** For this purpose, ninety-six days-old barred male chicks, weighing 28.33 g in average were randomly divided into 12 groups of 8 chicks each. Three feeding programs (FP) each comprising a starter diet from day old to 12 weeks (S0: 2800 kcal kg^{-1}, S1: 2900 kcal kg^{-1}, S2: 3000 kcal kg^{-1}) and a grower diet from 13-20 weeks (G0: 2900 kcal kg^{-1}, G1: 3000 kcal kg^{-1}, G2: 3100 kcal kg^{-1}) were used and designated FP1, FP2 and FP3, respectively. Each of the FP was randomly distributed to 4 groups of 8 birds in a completely randomized design. At 20 and 30 weeks of age, 8 cocks from each treatment group were randomly slaughtered to determine the testes weight, the testicular shape index and the testes stereological and histological traits. **Result:** At 20 weeks of age, feeding programs FP0 and FP2 showed a significant increase ($p<0.05$) in the relative weight of testes, the seminiferous tubule area and diameter, the seminiferous tubule lumen area and diameter and the germinal cells thickness as compared to FP1. Focal foci were present in seminiferous epithelium of cock fed on FP0 and FP2, while they were absent in testes of cock treated with FP1. At 30 weeks of age, the seminiferous tubule lumen area was significantly higher ($p<0.05$) with FP0 as compared to values obtained with FP1 and FP2; meanwhile, germinal cells thickness was significantly higher ($p<0.05$) with FP1. The number and volume of foci were less important in testes of cocks fed on FP1 as compared to testes of cocks which received FP0 and FP2. **Conclusion:** In conclusion, the diet energy level of 2800 kcal kg^{-1} during the starter phase and of 2900 kcal kg^{-1} during the grower stage improved the testes histological and stereological traits of indigenous barred male chicken.

Key words: Diet energy, histological characteristics, male chicken, reproductive function, testes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In birds and mammals, nutrition and particularly energy metabolism strongly influence the reproductive functions[1]. In birds, testes development, semen production and quality and fertility have been reported to be influenced by diet energy level[2-4].

According to Kirby *et al*.[5], an increase in calorie intake may lead to an increase in testes weight, while others pointed out that an increase in energy intake during starter phase may lead to precocious development and early regression of testes in poultry[2,4]. Inversely, an energy deficiency delays the testicular development[6,7]. However, reports on the effect of diet energy level on histological traits are rare. Nevertheless, Briere *et al*.[7] hypothesized that an increase in energy intake induces an alteration of functional state of stem spermatogonia, thus decreasing their ability to develop into spermatocyte I. The effect of energy on testes development may result in an increase or decrease of the number and/or size of seminiferous tubules or in a testes deposition of fat. In bulls fed with a high energy level diet, Swanepoel *et al*.[8] pointed out an increase in the number of seminiferous tubules showing severe atrophy. In areas of atrophy, important condensation of reticulin fibers and collagen laced most of the layers of reproductive organs. On the other hand, these authors reported important fat deposition in testes of bulls fed with a high energy level diet compared to medium energy and low energy diets. In chicken, Briere *et al*.[7] reported an increase in intra-testicular concentration of cholesterol. Seminiferous tubule size is positively correlated with the number and size of Sertoli cells which support and nourish a limited number of germ cells[9,10]. Guibert *et al*.[10] hypothesized that factors that can affect Sertoli cells proliferation in chicken could limit germ cells production and testes development. Tartarin *et al*.[11] showed that a positive energy balance in prepubertal testes of chicken may decrease Sertoli cells proliferation while stimulating their differentiation. However, further investigations are needed to elucidate the relationship between diet energy level and testicular development in cock.

Therefore, the aim of the present study was to determine the effect of dietary energy level on the histological traits of the cock testes.

MATERIALS AND METHODS

This study was carried out at the Teaching and Research Farm of the University of Dschang located between latitude 5°-7° North and Longitudes 8°-12° East. Dschang is located in the Western Sudano guinean Savanna of Cameroon at 1500 m above sea level. Mean wind speed is 1.60 m sec^{-1}; mean temperature is 20°C and relative humidity generally exceeds 60%. Annual rainfall varies between 1910 and 2010 mm. The raining seasons goes from mid-March to mid-November and the dry season from mid-November to mid-March.

A total of 96 local barred cocks, day old, with an average weight of 26-33 g were used. They were randomly divided into 12 groups of 8 cocks each. Cocks were housed in cages made of "raphia" bamboo and kept under similar environmental and managerial conditions during experiment. Feed and water were given *ad libitum* in adapted equipments.

Three feeding programs (FP0, FP1 and FP2) each comprising a starter diet from day old to 12 weeks of age (S) and grower diet from 13-30 weeks of age (G) were used as reported in Table 1. During starter or grower stage, diets were

Table 1: Percentage of ingredients and calculated chemical components of experimental diets

	Feeding program 0		Feeding program 1		Feeding program 2	
	S0 (2800 kcal)	G0 (2900 kcal)	S1 (2900 kcal)	G1 (3000 kcal)	S2 (3000 kcal)	G2 (3100 kcal)
Corn meal	42.00	47.00	48.00	49.00	43.00	45.00
Wheat bran	22.50	22.00	16.50	22.00	20.00	22.00
Cotton seed						
Cake	8.00	9.00	8.00	8.00	8.00	6.00
Soybean cake	15.00	11.00	15.00	7.00	15.00	13.00
Fish meal	6.00	3.00	6.00	6.50	6.00	3.00
Bone meal	0.00	2.00	0.00	1.50	0.00	1.50
Shellfish						
Powder	1.50	5.00	1.50	5.00	1.50	5.00
Premix 5% (*)	5.00	0.00	5.00	1.00	5.00	3.50
Palm oil	0.00	1.00	0.00	0.00	1.50	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	23.66	20.78	23.27	20.70	23.43	20.34
ME (kcal/kg)	2803.90	2936.03	2913.73	3013.51	3006.17	3100.95
Calcium (%)	1.49	1.74	1.48	1.51	1.49	1.55
Phosphorus (%)	0.76	0.74	0.69	0.73	0.71	0.74
Lysine (%)	1.31	1.06	1.29	1.10	1.49	1.06
Met (%)	0.43	0.40	0.43	0.40	0.43	0.46

*Premix 5%: Crude protein: 40%, Metabolizable energy: 2078 kcal kg^{-1}, Calcium: 8%, Phosphorus: 2.05%, Lysine: 3.30%, Met (Methionine): 2.40%, ME: Metabolizable energy different only by their energy density. During starter and grower phases respectively, each of the three diet energy level was randomly distributed to 4 groups of 8 birds in a completely randomized design.

At 20 and 30 weeks of age, 8 cocks from each treatment group were randomly slaughtered to determine the absolute and relative testes weight, the testicular shape index and the histological traits of testes (seminiferous tubules area and diameter, lumen area and diameter, germinal cell thickness). These histological parameters were determined using the software package Image_J.1.32j 0.0.0.0. For histological observation, Leica digital microscope-camera was used at 40× magnification.

Data collected were subjected to one-way analysis of variance (ANOVA) at significance level of $p<0.05$ following the General Linear Model procedure. When differences were significant between means, Duncan multiple range test was used to separate means. Pearson correlation coefficient between parameters was also performed at significance level of $p<0.05$. All statistical analyses were performed using SPSS 20.0.

RESULTS

Effect of dietary energy level on steorological traits of local barred cock testes: Results revealed that diet energy level had a significant effect on absolute and relative weight of testes and on seminiferous tubules measurements ($p<0.05$). Inconsiderate of age, feeding program FP1 (2900 kcal kg^{-1} in starter and 3000 kcal kg^{-1} in grower diet) significantly decreased ($p<0.05$) the absolute weight of testes compared to other feeding programs (Table 2). At 20 weeks of age, FP0 (2800 kcal kg^{-1} in starter and 2900 kcal kg^{-1} in grower diet) and FP2 (3000 kcal kg^{-1} in starter and 3100 kcal kg^{-1} in grower diet) significantly increased ($p<0.05$) the relative weight of testes, the seminiferous tubule area and diameter, the seminiferous tubule lumen area and diameter and the germinal cells thickness as compared to feeding program FP1 (Table 2). At 30 weeks of age, the seminiferous tubule lumen area was significantly higher ($p<0.05$) with FP0 as compared to other feeding programs (FP1 and FP2); germinal cells thickness was significantly higher ($p<0.05$) with FP1 as compared to other feeding program (Table 2). Although, absolute weight of testes was significantly lower ($p<0.05$) with FP1 compared to FP2, seminiferous tubules measurements were relatively higher with FP1. Except for seminiferous tubule lumen area which decreased significantly from 20-30 weeks with FP2 and germinal cells thickness which increased

Table 2: Effect of dietary energy level on absolute and relative weight of testes and seminiferous tubules measurements (Mean±SEM) of local barred cocks

	20 weeks			30 weeks		
Traits	FP0 (2800-2900 kcal kg^{-1})	FP1 (2900-3000 kcal kg^{-1})	FP2 (3000-3100 kcal kg^{-1})	FP0 (2800-2900 kcal kg^{-1})	FP1 (2900-3000 kcal kg^{-1})	FP2 (3000-3100 kcal kg^{-1})
Absolute weight of testes (g)	13.715 ± 2.82^{a}	5.706 ± 1.54^{b}	15.615 ± 0.506^{a}	19.106 ± 2.31^{a}	12.244 ± 0.97^{b}	17.709 ± 0.67^{a}
Relative weight of testes (%)	0.745 ± 0.147^{aA}	0.250 ± 0.045^{bA}	0.935 ± 0.044^{aA}	0.994 ± 0.08^{aA}	0.924 ± 0.094^{aB}	1.034 ± 0.021^{aA}
Shape index of testes	0.580 ± 0.013^{aA}	0.586 ± 0.02^{aA}	0.576 ± 0.009^{aA}	0.594 ± 0.011^{aA}	0.622 ± 0.007^{aA}	0.625 ± 0.019^{aB}
Seminiferous tubules area (µm^2)	37139.910 ± 2540.15^{aA}	18398.180 ± 1005.62^{bA}	36104.590 ± 3094.83^{a}	46715.910 ± 2841.73^{aB}	40343.64 ± 2493.74^{aB}	38795.170 ± 3755.99^{a}
Seminiferous tubules diameter (µm)	352.420 ± 14.228^{a}	256.170 ± 10.999^{bA}	329.539 ± 19.716^{a}	381.710 ± 19.752^{a}	372.04 ± 15.831^{aB}	370.880 ± 30.929^{a}
Seminiferous tubules lumen area (µm^2)	6986.240 ± 471.453^{aA}	2573.300 ± 218.543^{bA}	7079.510 ± 727.614^{aA}	10947.440 ± 599.78^{aB}	7276.48 ± 855.76^{bB}	6273.620 ± 638.33^{bB}
Seminiferous tubules lumen diameter (µm)	208.450 ± 11.35^{aA}	152.950 ± 16.753^{bA}	197.298 ± 17.853^{aA}	272.520 ± 16.335^{aB}	231.63 ± 14.137^{aB}	236.640 ± 24.296^{aB}
Germinal cells thickness (µm)	41.950 ± 0.98^{a}	30.300 ± 0.73^{bA}	42.610 ± 1.97^{a}	40.360 ± 0.76^{a}	47.69 ± 1.44^{bB}	42.570 ± 0.62^{c}

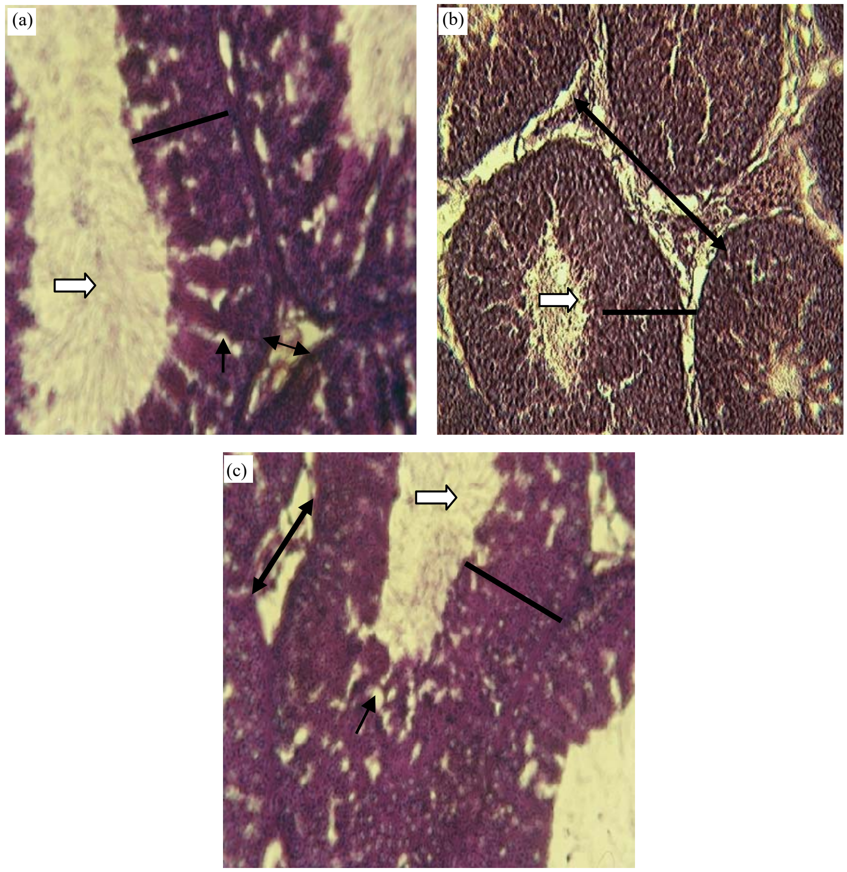

Fig. 1(a-c): Cross section of the left testes of cocks at 20 weeks (HE×400). (a) Testes of cocks fed on FP0, (b) Testes of cocks fed on FP1 and (c) Testes of cocks fed on FP2

(–) Germinal cells thickness, (⇒) Seminiferous tubules lumen, (↔) Interstitial space, (←): Foci of seminiferous epithelium

significantly only with FP1, the other traits increased irrespective of feeding program. However, the increase was significantly more important (p<0.01) with FP1 and less important with FP2.

Effect of diet energy level on histological traits of local barred cock testes: At 20 weeks of age, focal foci were present in seminiferous epithelium of cocks fed on FP0 (2800-2900 kcal kg^{-1}) and FP2 (3000-3100 kcal kg^{-1}) (Fig. 1a-c), meanwhile they were absent in testes of cocks treated with FP1 (2900-3000 kcal kg^{-1}) (Fig. 1b). Large interstitial spaces were also present in testes of cocks which received feeding program FP1 (Fig. 1b) as compared to spaces in testes of cocks treated with FP0 and FP2. Cross section of testes showed the highest density of spermatozoa in seminiferous lumen of cocks treated with FP0 (Fig. 1a).

At 30 weeks of age (Fig. 2a-c), the main observations were the presence of multiple foci in seminiferous epithelium of cocks, no matter the feeding program. However, the number and volume of those foci were less important in testes of cocks fed on FP1 as compared to those fed on FP0 and FP2. Meanwhile, Fig. 2a and c show low spermatozoa density in seminiferous lumen of cock fed with FP0 and FP2 as compared to density observed respectively in Fig. 1a and c at 20 weeks of age.

Whatever the age and diet energy level, no round cells were found in the seminiferous tubule lumen.

DISCUSSION

At 20 weeks of age, the heaviest testes showed highest values of seminiferous tubules measurements. It is well established that testicular growth and development reflect the increase of histological traits such as the seminiferous tubules diameter and area[6,9]. At the same age, feeding programs FP0 and FP2 significantly increased seminiferous tubules measurements as compared to FP1. These results suggest that FP0 and FP2 accelerated sertoli and germinal

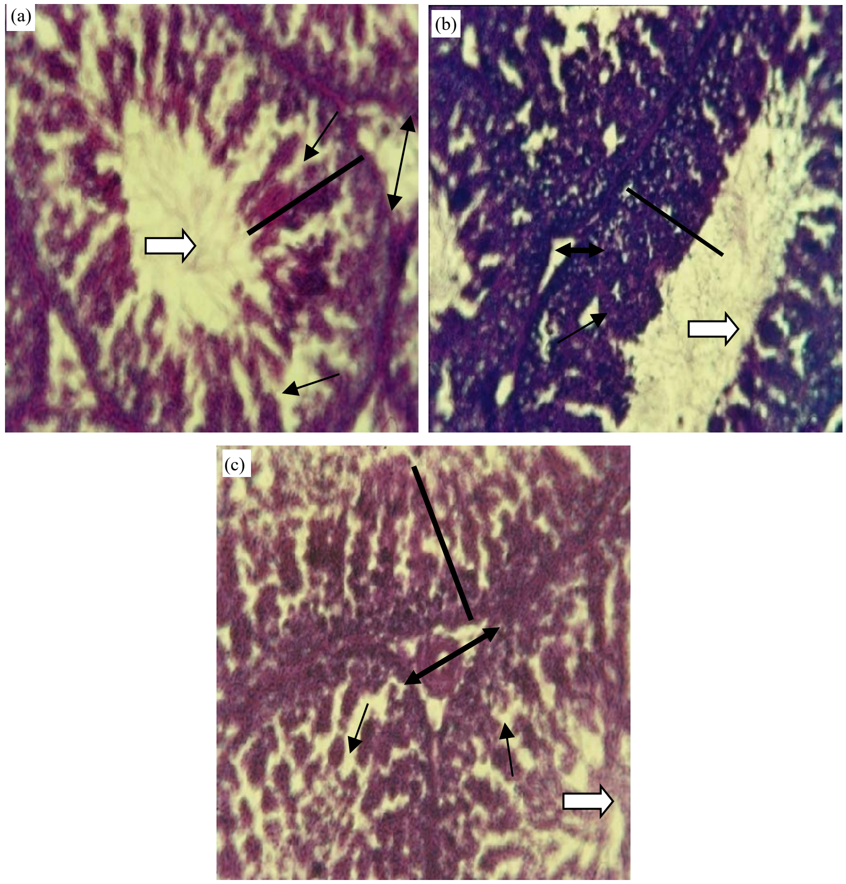

Fig. 2(a-c): Cross section of the left testes of cocks at 20 weeks (HE×400). (a) Testes of cocks fed on FP0, (b) Testes of cocks fed on FP1 and (c) Testes of cocks fed on FP2

(–) Germinal cells thickness, (⇨) Seminiferous tubules lumen, (↔) Interstitial space, (←) Foci of seminiferous epithelium

cells proliferation and maturation. As compared to FP0, diets energy level of FP1 may be higher than the cock's requirements. In fact, birds eat essentially to cover their energy needs. So, a hyper energetic diet may not cover other nutritive needs such as protein, vitamins or minerals. The resulting nutrient deficiency may negatively affect growth and testicular development[12,13]. Although, FP2 has higher energy level than FP1, it seems to have covered nutrients requirements and increased testicular development. Actually, FP2 diets were formulated with palm oil. Palm oil binds small particles, increase chicken appetite, feed consumption of FP2 diets and so nutrients intake.

Focal foci present in seminiferous epithelium of cocks fed on FP0 and FP2 can be explained by apoptosis of sertoli cells. Such apoptosis of sertoli cells has been reported by Deviche *et al.*[9] during reproductive season in bird.

From 20-30 weeks of age, seminiferous tubule lumen area significantly increased with treatments FP0 and FP1 but decreased significantly with treatment FP2. The increase of seminiferous tubule lumen area is normal. In fact, from pubertal to adult phases, testicular development is associated with the increase of seminiferous tubule traits and their activities. For treatment FP2, the decrease may be attributed to a fat deposit in interstitial space which would have limited testicular development. Although, absolute weight of testes significantly increased with treatment FP2 from 20-30 weeks, neither seminiferous tubules area nor diameter increased. Swanepoel *et al.*[8] pointed out important fat deposition in testes of bulls fed with high energy diet compared to medium and low energy diet. On the other hand, Briere *et al.*[7] reported that in chicken, high energy intake leads to an increase of intra testicular concentration of cholesterol. Meanwhile, Kirby *et al.*[5] reported that an increase in caloric intake may lead to an increase in testes weight, without improving spermatogenic efficiency.

The presence of large vacuole with treatment FP0 and FP2 expressing important sertoli cells apoptosis which confirmed the findings of Romero-Sanchez *et al.*[2] and Briere *et al.*[7] who reported that precocious testes development is associated with precocious regression of testes. The absence of round cells in seminiferous tubule lumen suggests that dietary energy level did not affect spermiogenesis.

CONCLUSION

From this study, it can be concluded that the diet energy level of 2800 kcal kg^{-1} during starter phase and the diet energy level of 2900 kcal kg^{-1} during grower phase improved the histological and stereological traits of testes of indigenous barred male chicken found in the western highlands of Cameroon.

SIGNIFICANCE STATEMENT

Improving the productivity of indigenous chicken is now a major concern in developing countries. For this purpose, projects on determining the nutritional requirement for optimal production have been implemented. This study determines the diet energy level requirement for better reproductive performances and growth of barred chicken. From this study, the recommended diet energy level can be used as basis to determine other nutrient requirements for local barred cock. On the other hand, this study contributes to the understanding of the underlying mechanism by which high energy diet induces early testicular regression in cock.

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