



Influence of Varying Levels of Dietary Energy and Protein on Testicular Histomorphometry of Caged FUNAAB – Alpha Cocks

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Abstract

This study was designed to evaluate the effect of varying levels of dietary energy and protein on testicular morphology and histology of FUNAAB-alpha cocks. A total of sixteen cocks, 32 weeks of age were divided into four treatment groups replicated four times each in two blocks in a Randomized Complete block Design. The birds were reared in individual cages. Four experimental diets; Control (2600 Kcal/kg and 13% CP), HELP (2800 Kcal/kg and 10% CP), HEHP (2800 Kcal/kg and 16% CP) and LEHP (2400 Kcal/kg and 16% CP) were formulated. Experimental diets and water were offered ad libitum throughout the experimental period. No significant difference was observed among treatment means for most of the parameters measured except weight of paired vas deferens. The HELP group had heavier ($P<0.05$) vas deferens compared to control and LEHP but HEHP had a similar weight to all the other groups. The histological sections of testes for the different dietary treatments revealed that testes in cocks fed the different diets were well developed with all series cells in place and showed no adverse effect on spermatogenesis. It was concluded that diets with metabolizable energy of 2600 Kcal/kg and crude protein as low as 13% could be fed to FUNAAB – alpha cocks that are to be used for semen collection and subsequent insemination.

KeyWords: Cocks, Histology, Nutrition, Reproduction, Testes morphology

Introduction

It is well known that dietary energy level affects body weight, sexual maturity, semen production and quality in the cock (Romero-Sanchez *et al.*, 2008; Ghonim *et al.*, 2010; Tadondjou *et al.*, 2013). Kirby *et al.* (1996) also noted that increase in calorie intake may lead to an increase in testes weight with no apparent increase in spermatogenic efficiency. Similarly, Tadondjou *et al.* (2013) reported that high (3000 and 3100 Kcal/kg) and low (2800 and 2900 Kcal/kg) dietary energy levels during chick and grower phase lead to an increase in testes weight of cocks compared to medium energy (2900 and 3000 Kcal/kg). They further suggested that high energy intake lead to advanced testicular development and testicular hyper-trophy.

The role of crude protein (CP) in the reproduction of roosters is well recognized (Zhang *et al.* 1999). The authors showed that birds on 12 to 16% CP before sexual maturity did not differ in testes weight,

sperm concentration, semen volume and duration of semen production. In another experiment, Hocking and Bernard (1997) reported that semen production was not better in caged broiler breeders fed 16% CP than 12% during 21 to 66 weeks of age and feed increment had no effect on the semen production. Romero-Sanchez *et al.* (2007) compared 12 and 17% CP content in diet on fertility of male breeders from 27 to 64 weeks and concluded that the 12% CP diet improved both weekly and cumulative fertility.

In a breeder flock, fertility rates are often related to the male performance. However, their dietary management is often inadequate as they are fed diets formulated to meet the needs of the females (Gonçalves *et al.* 2015). Hocking (1989) reported that roosters fed diets meant for females (high protein) experience early decline in fertility rate. Similarly, excessive intake of calcium by cocks has been linked to decreased performance mainly due to interference with

inorganic manganese, zinc and magnesium uptake, making them unavailable for absorption (Waldroup, 1996).

In Nigeria efforts have been geared towards the development of indigenous chicken breeds with improved meat and egg production. These efforts have led to the development of the Shika brown layers by National Animal Production Institute (NAPRI) (Ikanni and Annatte, 2000) and the FUNAAB – alpha by the Federal University of Agriculture Abeokuta (FUNAAB) (Adebambo, 2015). However, there is little information on the nutritional requirements of cocks of these strains as it affects testicular histomorphology. The aim

of the present study was therefore to evaluate the effect of dietary energy and protein levels on testicular morphology and histology of FUNAAB-alpha cocks.

Materials and Methods

A total of 16 cocks, aged 32 weeks, were divided into four treatment groups replicated four times each in two blocks in a Randomized Complete Block Design. The birds were reared in individual cages in a two tier-cage. Each tier represented a block. Semen was collected from each cock once a week. Experimental diets (Table 1) and water were offered *ad libitum* throughout the 34-week experimental period.

Table 1: Ingredients and Percentage Composition of Different Energy and Protein Diets Fed to Cocks

Ingredients	CONTROL	HELP	HEHP	LEHP
Maize	62.21	74.69	66.66	49.09
Soya bean meal	7.20	1.18	18.89	14.60
Wheat bran	26.79	20.33	10.65	32.51
Bone meal	1.75	1.75	1.75	1.75
Limestone	1.25	1.25	1.25	1.25
Salt	0.35	0.35	0.35	0.35
Premix*	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Total	100	100	100	100
Calculated Analysis				
ME (Kcal/kg)	2600	2800	2800	2400
Crude Protein (%)	13.0	10.0	16.0	16.0
Calcium (%)	1.06	1.04	1.08	1.09
Phosphorus (%)	0.79	0.70	0.70	0.88
Proximate Composition				
Dry matter (%)	89.18	89.75	89.82	88.95
Crude protein (%)	13.22	10.34	16.19	16.04
Ether extract (%)	2.66	4.11	3.94	2.15
Crude fibre (%)	6.96	5.15	4.04	9.79
Ash (%)	7.87	7.44	7.71	8.01
NFE (%)	58.47	62.71	57.94	52.96
ME (Kcal/kg)	2606.69	2802.98	2807.79	2507.43

Control – Standard diet, HELP – High energy low protein, HEHP – High energy high protein, LEHP – Low energy High protein, NFE – Nitrogen free extract, ME – Metabolizable energy (Calculated)

*Each 2.5kg HI-Mix[®] vitamin/mineral premix contain; Vitamin A – 10,000,000 I.U, Vitamin D3 – 2,000,000 I.U, Vitamin E – 12,000mg, Vitamin K3 – 2,000mg, Vitamin B1 – 1,500mg, Vitamin B2 – 4,000mg, Vitamin B6 – 1,500mg, Niacin – 15,000mg, Vitamin B12 – 10mcg, Pantothenic Acid – 5,000mg, Folic Acid – 500mg, Biotin – 20mcg, Choline Chloride – 100,000mg, Manganese – 75,000mg, Zinc – 50,000mg, Iron – 20,000mg, Copper – 5,000mg, Iodine – 1000mg, Selenium – 200mg, Cobalt – 5,000mg, Antioxidant – 125,000mg.

At the end of the experiment, paired testes were collected from each cock, trimmed of all adhering fat and tissues and weighed to the nearest 0.01g using an electronic scale (SF – 400). Testis length and width were measured using a digital Vernier caliper to the nearest 0.01 mm (Bath and Chaudhari, 2002). Volume was obtained using Archimedes principle. This involved, using a measuring cylinder containing known quantity of water, the testis was gently dropped in and the volume of water displaced was recorded as the volume (Shil *et al.* 2015).

The Vas deferens was trimmed of all adhering tissues and the paired weight obtained using an electronic scale while the length was measured to the nearest millimeter with the aid of a ruler.

Testicular samples were taken from all the sixteen cocks in the experiment for histology. The samples were fixed in 10% formal saline for one week and thereafter processed for routine paraffin histological sectioning. The tissues were dehydrated through graded concentration of ethanol (70%, 90%, absolute ethanol) and cleared in xylene. The tissues were pre-impregnated in xylene paraffin wax in the oven and embedded in pure paraffin wax. The organs were sectioned at 7µm thickness and tissues were stained with Haematoxylin and Eosin (H and E) for light microscopic examinations (Bancroft and Stevens, 1972). Tissues were mounted on wooden chocks and cooled in the refrigerator at 4°C. The mounted tissues were exposed by trimming

and sectioned at 5µm thickness. The tissues were picked on egg albumin smear glass slides and dried in the oven at 45°C. The organs sectioned at 5µm thickness were stained with Masson Trichrome and Haematoxylin and Eosin (H and E) for light microscopic examinations (Baker and Silverton, 1985).

To obtain the diameter of the seminiferous tubules of testis, five vertical sections from the polar and equatorial regions were sampled and unbiased numerical estimations were carried out (Qin and Lung, 2002; Gundersen and Jenson, 1987). The diameters (D) of seminiferous tubules with profiles that were round or nearly round were measured and the mean diameter determined as the average of the five readings.

Analysis of variance was carried out on the recorded data using the General Linear Model sub routine of SPSS 20.0 (2011). Where means differed, Duncan Multiple Range Test (DMRT) was used to separate them.

Results

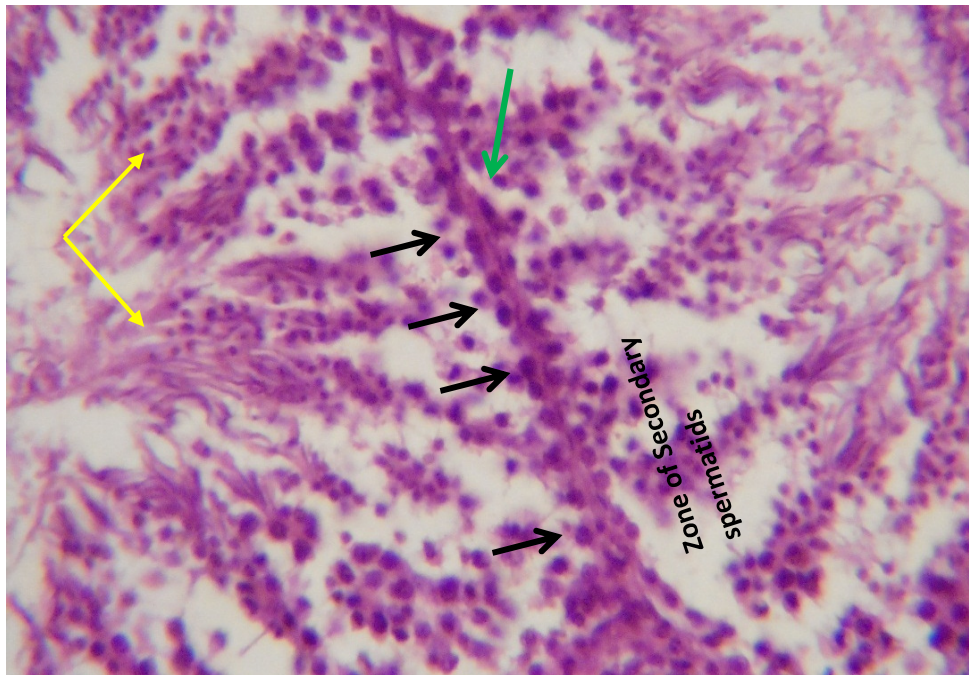
The effects of dietary treatments on histomorphology of roosters of the FUNAAB –alpha are shown in Table 2. There was no significant difference among treatment means for most of the parameters measured except weight of paired vas deferens. The HELP group had heavier vas deferens as compared to control and LEHP but HEHP had similar weight to all the other groups.

The histological sections of testes for the different dietary treatments are shown on Plates I to IV. The testes of cocks fed diets control, HELP, HEHP and LEHP were well developed with all series cells in place and showed no adverse effect on spermatogenesis with numerous spermatozoa in the tubular lumen.

Table 2: Effect of Dietary Energy and Protein on Testis Morphology of Caged FUNAAB - Alpha Cocks

Parameter	CONTROL	HELP	HEHP	LEHP	± SEM	p-Value
Live weight (g)	3300	3175	3375	3175	902.50 ^{NS}	0.99
Paired testes weight (g)	20.9	25.7	21.8	19.6	6.31 ^{NS}	0.88
Right testis weight (g)	10.6	11.7	8.13	9.29	2.63 ^{NS}	0.67
Left testis weight (g)	10.4	13.9	11.2	9.57	3.67 ^{NS}	0.76
Gonadosomatic index (%)	0.69	0.61	0.64	0.81	0.15 ^{NS}	0.59
Right testis length (mm)	40.4	39.9	36.5	34.9	11.13 ^{NS}	0.97
Left testis length (mm)	39.9	42.7	40.1	40.3	6.07 ^{NS}	0.92
Right testis width (mm)	20.1	19.5	17.2	24.0	5.45 ^{NS}	0.98
Left testis width (mm)	19.9	19.9	19.4	19.9	2.12 ^{NS}	0.52
Paired testes volume (ml)	17.7	27.0	18.3	17.5	7.29 ^{NS}	0.58
Right testis volume (ml)	9.00	13.0	7.75	12.0	4.42 ^{NS}	0.53
Left testis volume (ml)	8.75	14.0	10.5	10.0	3.48 ^{NS}	0.54
Testes density (gml ⁻¹)	1.19	1.20	1.70	0.97	0.20 ^{NS}	0.69
Weight of vas deferens (g)	2.20 ^b	4.38 ^a	2.48 ^{ab}	2.00 ^b	0.92 [*]	0.12
Length of vas deferens (mm)	152	148	137	149	18.21 ^{NS}	0.76
Tubular diameter (µm)	41.4	39.5	38.3	42.8	2.63 ^{NS}	0.27

Control- Standard Diet, HELP- High Energy Low Protein, HEHP- High Energy High Protein, LEHP- Low Energy High Protein

^{a, b, c, ...} Means within the same row bearing different superscripts differ significantly ($p < 0.05$); SEM: standard error of mean; NS: non significant ($p > 0.05$); *: Significant ($p < 0.05$).**Plate I:** Photomicrograph of chicken testis fed Standard Diet showing presence of spermatozoa, turfs spermatids (yellow arrows), spermatogonia (black arrows) and interstitial connective tissues (green arrow) (Haematoxylin and Eosin) x400

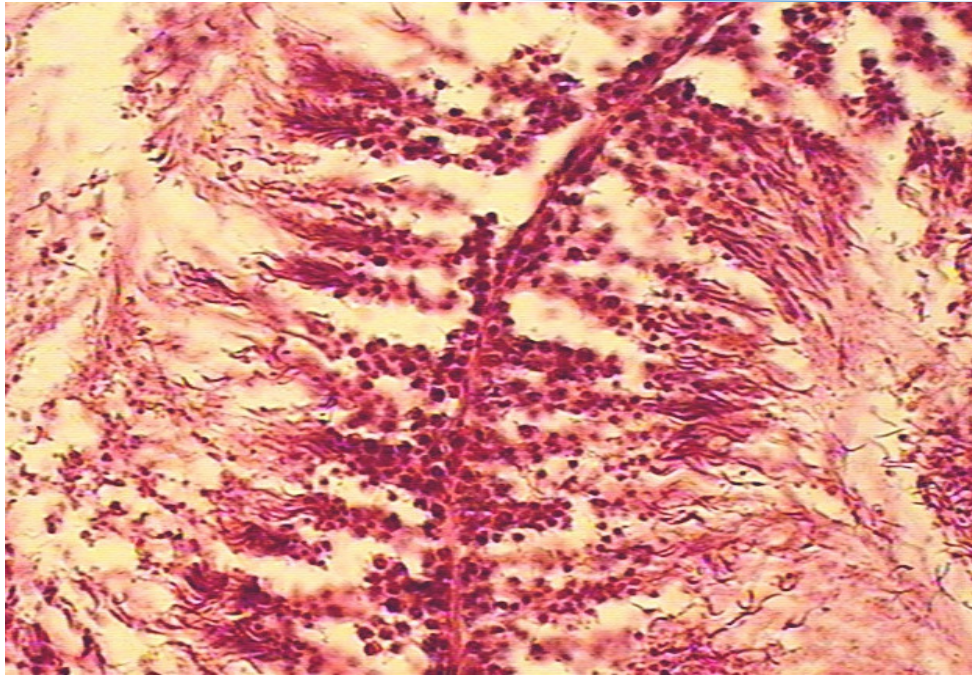


Plate II: Photomicrograph of chicken testis fed High Energy Low Protein diet showing normal features with lots of spermatozoa in the seminiferous tubules (Haematoxylin and Eosin) x400

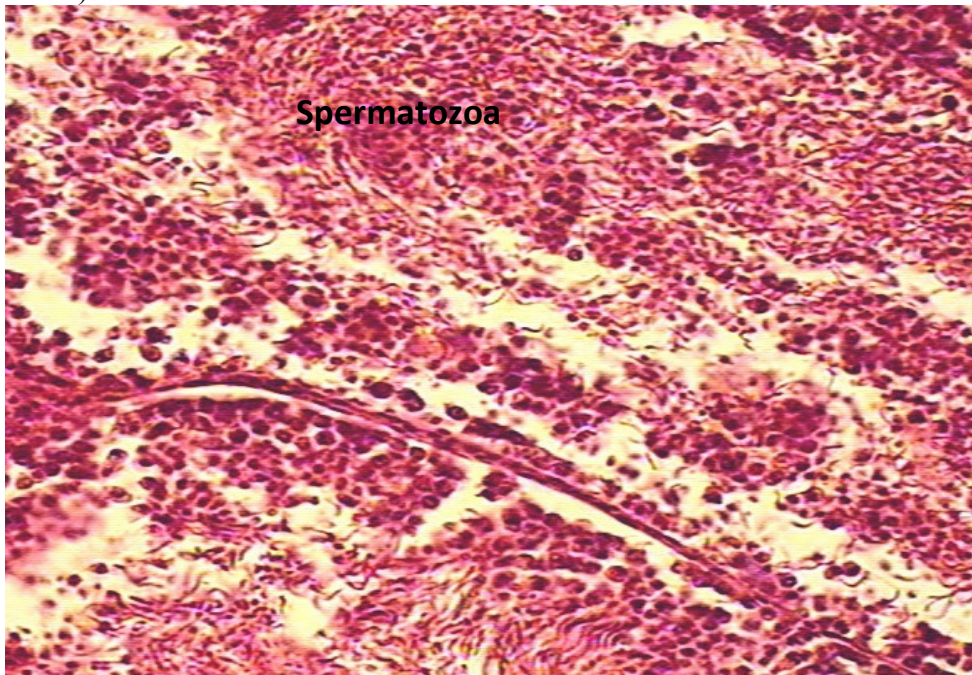


Plate III: Photomicrograph of chicken testis fed High energy High Protein diet showing normal features with lots of spermatozoa in the seminiferous tubules (Haematoxylin and Eosin) x400

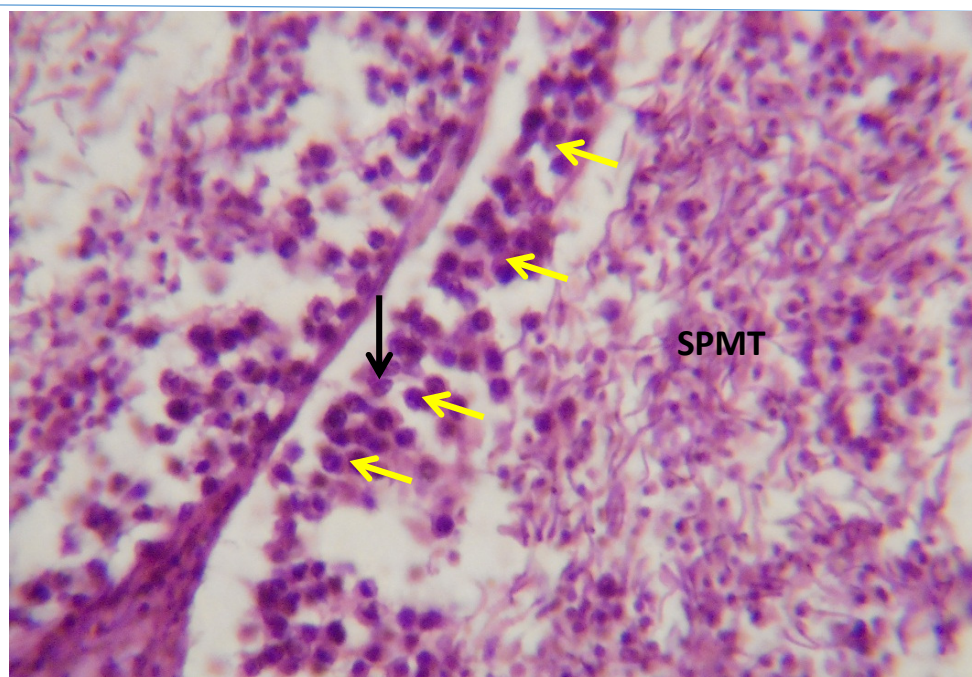


Plate IV: Photomicrograph of chicken testis fed Low Energy High Protein showing presence of spermatozoa mixed with tertiary spermatids (SPMT), spermatogonia (yellow arrows) and interstitial connective tissues (black arrow) (Haematoxylin and Eosin) x400

Discussion

Individual and paired testes weights were not influenced by dietary treatment. In a similar observation, Zhang *et al.* (1999) reported that when roosters were fed either 12 or 16% crude protein, testes weight did not differ. Similarly, Fontana *et al.* (1990) were of the opinion that weights of the testes were more closely associated with body size than with the level of dietary protein.

The HELP combination had the heaviest paired vas deferens followed by the HEHP diet. It seems high energy in the diet supports high vas deferens weight. The weight of vas deferens (2.0 to 4.38 g) observed in this study is higher than 1.6 and 0.96 to 2.0 g reported by Bath and Chaudhari (2002) and Urom (2016), respectively for the Nigerian local chicken. The higher weight in this study may be because the FUNAAB-Alpha cocks have been selected for improved growth as compared to the local strains used by the authors. The length of Vas deferens is however, similar to 9.4 to 14.70 cm reported by Bath and Chaudhari (2002). The size of

the testes, epididymis and vas deferens are related to semen output. Thus birds with large reproductive organs will likely produce higher quantity of semen (Urom 2016).

Histological section of testes from all the experimental groups showed normal features with lots of spermatozoa. This is similar to the observations of Ozegbe and Aina (2012) who reported that the seminiferous tubules of Japanese quail fed normal or low protein and energy did not show any observable lesions.

Conclusions

It was concluded that diets with metabolizable energy of 2600 kcal/kg and crude protein as low as 13% could be fed to FUNAAB – alpha cocks that are to be used for semen collection and subsequent insemination.

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