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Effects of varying dietary energy and protein levels on gross morphology and histology of testes of breeder FUNAAB – Alpha cocks

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Abstract

The effects of varying levels of dietary energy and protein on gross morphology and histology of testes of FUNNAB – Alpha chickens were studied at the Abubakar Tafawa Balewa University Bauchi, Bauchi state. Twelve cocks were randomly divided into four dietary treatments; Standard diet (SD) (Control) (2650Kcal/Kg ME/ 16%CP), High Energy – Low Protein (HELP, 2800 Kcal/Kg ME/ 14%CP), High Energy – High Protein (HEHP, 2800 Kcal/Kg ME/ 18% CP) and Low Energy – Low Protein (LEHP, 2400 Kcal/Kg ME/ 18%CP) groups. A total of twelve FUNAAB – Alpha cocks were used for this experiment. The cocks were reared in floor pens under natural mating. At 69 weeks of age, all the cocks were slaughtered and testicles carefully removed for gross morphology and histological studies. A significant (P<0.05) influence of diet was noted on live weight with cocks fed HELP diet being heavier (P<0.05) than those in the other treatment groups. Means for all parameters of testicles measured where not different (P>0.05) from each other. Histological sections of the testes showed that HELP diet had mild effect on spermatogenesis evidenced by the scanty spermatozoa in the tubular lumen. It was concluded that FUNAAB – alpha cocks can be fed diets meant for breeding hens without adverse effect on spermatogenesis. However, feeding the LEHP diet produced roosters that were overweighed with slightly impaired spermatogenesis.

Keywords: FUNAAB –alpha cock, spermatogenesis, Energy, Protein

Introduction

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). Obi *et al.* (2013) reported that nutrition is important in pre-puberty, puberty and maturity stages of male Broiler Breeders. They further observed that, malnutrition or over consumption lead to weight loss or gain respectively at any of the three sexual stages possibly affecting sperm production. This disruption can lead to permanent non-functional testis and reduced reproductive performance. It was also reported by Martin and Walkden-Brown (1995) that circulating nutrients may directly affect the secretion of luteinizing hormone (LH) in the pituitary or indirectly by decreasing signals from median eminence. Although it is well known that energy and protein are major nutrients that affect the reproductive performance of poultry, for the Nigerian local chicken there is a paucity of information on their nutritional requirements as it affects reproduction. Furthermore 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). Similarly, the Federal University of Agriculture Abeokuta (FUNAAB) has developed the FUNAAB – alpha (Adebambo, 2015). However, there is little information on the influence of nutrition on reproduction capability of these strains. This study was therefore designed to evaluate the effects of varying levels of dietary energy and protein on morphometry and histology of the testis of FUNAAB – alpha cock fed breeder hen diets.

Materials and methods

The experiment was conducted at a location about six kilometres from the Animal farm of the Abubakar Tafawa Balewa University Bauchi, Bauchi state, Nigeria. Bauchi State is located between latitudes 9° 3′ and 12° 3′ north, longitudes 8°50′ and 11° east of the equator, at an elevation of 537 meters above sea level(Anonymous, 2011; Ngu *et al.,* 2014). The vegetation is Sahel/Sudan in the north and guinea savannah in the central and western zones (Institute of Agricultural Research/Bauchi State Agricultural Development Program [IAR/BSADP], 1996).

A total of 12 FUNAAB – alpha cocks (35 weeks of age) were randomly divided into four dietary treatment groups with three replicates each in three blocks (on deep litter in conventional open sided house under natural day light)as follows; Standard diet for exotic layer (SD)(ME-2600Kcal/kg and CP- 16%)(Control), high energy-low protein (HELP) (ME- higher than SD by 7.7% and CP lower by 12.5%), high energy-high protein (HEHP) (ME-higher than SD by 7.7% and CP- higher by 12.5%) and low energy- high protein (LEHP) (ME- lower than SD by 7.7% and CP- higher by 12.5%) (Table 1). The standard diets were based on the recommendations of Olomu (2011) for exotic hens in the tropics.All diets were provided in mash form. The diets and water were given *ad libitum* throughout the experimental period. The cocks were reared with hens in a mating ratio of 1:5 (cock: hen). At 69 weeks of age, all the cocks were slaughtered and testicles carefully removed for gross morphology and histological studies.

Testes collected were trimmed of all adhering fat and tissue 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. 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 millimetre with the aid of a ruler.

Testes were taken from all the sixteen cocks in the experiment for histology. They 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

Table1: Ingredients and percentage composition of different energy and protein diets fed to breeder chickens

Ingredients	SD	HELP	HEHP	LEHP
Maize	56.83	68.34	58.31	45.33
Soya bean meal	20.61	16.28	28.55	24.95
Wheat bran	12.01	4.82	1.59	19.19
Vegetable oil	-	-	1.00	-
Bone meal	2.75	2.75	2.75	2.75
Limestone	7.00	7.00	7.00	7.00
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	100.00	100.00	100.00	100.00
Proximate Composition				
Dry matter (%)	91.34	90.99	90.13	91.11
Crude protein (%)	16.13	13.95	18.19	18.05
Ether extract (%)	3.05	4.59	4.95	3.19
Crude fibre (%)	8.11	4.27	3.64	10.75
Ash (%)	14.27	14.2	14.65	14.75
NFE (%)	49.78	53.98	48.9	44.37
ME (Kcal/Kg)	2613.49	2807.902	2806.79	2503.927

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

*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.

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 equitorial regions were sampled and unbiased numerical estimations were carried out (Gundersen and Jenson, 1987; Qin and Lung 2002). 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.

Data was subjected to analysis of variance using the general linear model of SPSS 20.0 (2011) and where means differed, Duncan multiple range test (DMRT) was used to separate them (Duncan, 1955).

Results

Table 2 shows the effect of varying levels of dietary energy and protein on live weight and morphology of testes of FUNAAB – alpha cocks reared on deep litter under natural mating conditions.

Cock live weight for SD, HELP, HEHP and LEHP were 3083, 4550, 2816 and 2600g respectively. A significant ($P<0.05$) influence of diet was noted on live weight with cocks fed HELP diet being heavier ($P<0.05$) than those in the other treatment groups.

Means for all parameters of testicles measured where not different ($P>0.05$) from each other. Paired testes weight ranged between 21.21 to 33.93g while volume was 20.37 to 32.33. Testes density was 1.05,

Table 2: **Effect of Dietary Energy and Protein on Testis Morphology of FUNAAB - alpha Chicken**

Parameter	SD	HELP	HEHP	LEHP	± SEM
Live Weight (g)	3083.00[b]	4550.00[a]	2816.67[b]	2600.00[b]	365.08*
Paired Testes Weight (g)	21.21	33.93	25.93	23.01	10.95[NS]
Left Testis Weight (g)	10.60	15.74	12.56	11.33	5.01 [NS]
Right Testis weight (g)	10.58	18.23	13.34	11.56	5.99 [NS]
Gonadosomatic Index (%)	0.69	0.79	0.92	0.88	0.29[NS]
Left Testis Length (mm)	38.42	44.32	42.87	39.53	4.21 [NS]
Right Testis Length (mm)	40.62	45.57	35.32	39.54	5.87 [NS]
Left Testis Width (mm)	21.44	23.28	21.59	22.47	3.61 [NS]
Right Testis Width (mm)	20.37	23.45	23.09	21.34	3.89 [NS]
Paired Testes Volume (ml)	20.17	32.33	23.33	22.67	10.24 [NS]
Left Testis Volume (ml)	10.17	15.67	12.00	11.33	5.47 [NS]
Right Testis Volume (ml)	10.00	16.67	11.33	11.33	4.85 [NS]
Testes Density ($gm\ell^{-1}$)	1.05	1.04	1.13	1.02	0.09[NS]
Tubular Diameter (µM)	32.59	28.13	28.78	30.09	2.34[NS]

SD- Standard Diet, HELP- High Energy Low Protein, HEHP- High Energy High Protein, LEPH- 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: not significant (P>0.05); *: Significant (P<0.05).

1.04, 1.13 and 1.02$gm\ell^{1}$ for SD, HELP, HEHP and LEHP respectively. Means for tubular diameter (µM) for SD, HELP, HEHP and LEHP are 32.59, 28.13, 28.78 and 30.09 respectively. No significant (P>0.05) effect of dietary treatment was observed on this values.

Plates 1 to 4 show histological section of testes of the different treatments. The testes in cocks fed diets SD, HEHP and LEHP are well developed with all series cells in place and showed no adverse effect on spermatogenesis with numerous spermatozoa in the tubular lumen. However, HELP had mild effect on spermatogenesis evidenced by the scanty spermatozoa in the tubular lumen.

Discussion

Live weight of cocks was higher for birds on the HELP group while all the other groups had similar weights. However, dietary treatment had no effect on all gross morphological data of the testes taken. In an earlier study, Wilson *et al.* (1987) also indicated that broiler breeder males, fed protein levels ranging from 9 to 18%, showed no difference in testes weight at 53 weeks. Similarly, Fontana *et al.* (1990) reported that weights of the testes were more closely associated with body size than with the level of dietary protein.

Testes weight and gonadosomatic index of all the groups fall within the range of 9 – 30g and about 1% reported by Sturkie and Opel (1976) for chickens at sexual maturity. The range of gonadosomatic index recorded in this experiment was slightly lower than the 1.1% reported by Chidozie *et al.* (2010) for Nigerian local chickens. Gonadosomatic index indicates the efficiency of sperm production based on the assertion that testis weight is positively correlated to sperm production (Bath and Chaudhari, 2002; Fragoso *et al.,* 2013). Testicular length and width were slightly lower than values reported by Chidozie *et al.* (2010) but similar to that reported by

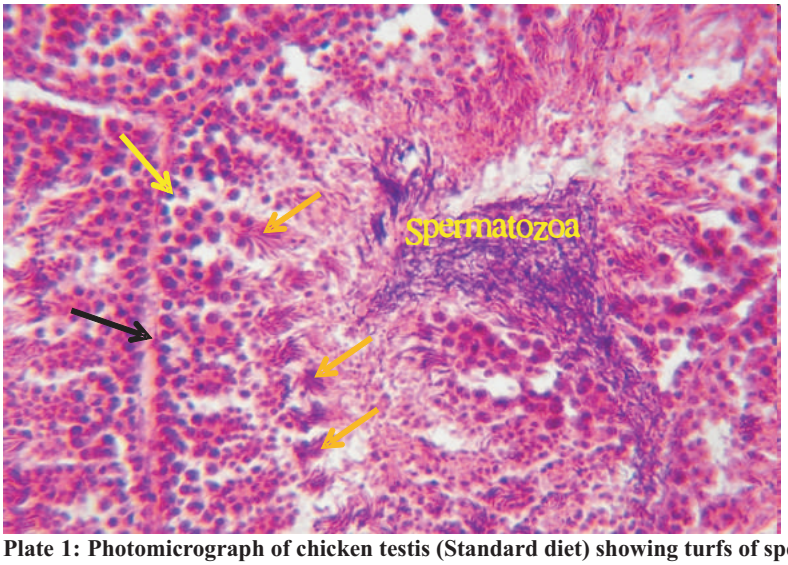

Plate 1: Photomicrograph of chicken testis (Standard diet) showing turfs of spermatids (orange arrows), spermatogonia (yellow arrow), interstitial connective tissue (black arrow) and spermatozoon in the lumen of the tubules H&E x400.

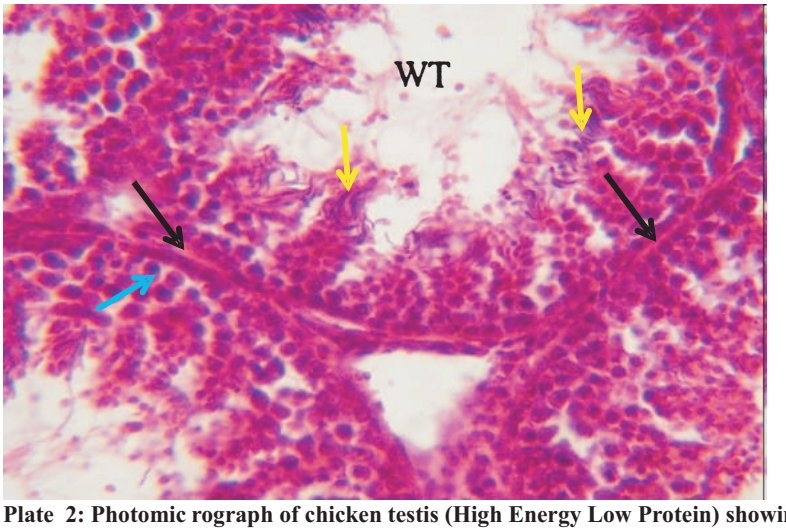

Plate 2: Photomic rograph of chicken testis (High Energy Low Protein) showing interstitial connective tissue (black arrow), few turfs of spermatids (yellow arrows) and mild depletion of spermatozoa in the tubular lumen (WT) H&E x400.

Obidi *et al.* (2008) for Shika brown breeder cocks.

The mean tubular diameters for the different treatment groups recorded in this study are lower than the average of 158.40µm reported by Chidozie *et al.* (2010) for the Nigerian local chicken and the range of 221.30 to 239.43µm observed by Adeyemo *et al.* (2007) for leghorn breeder cocks fed cottonseed cake. However, the observed values were higher than the 15.60µm reported by Onu and Ndodo (2003) for chickens. The observed difference can be attributed to breed and level of maturity of

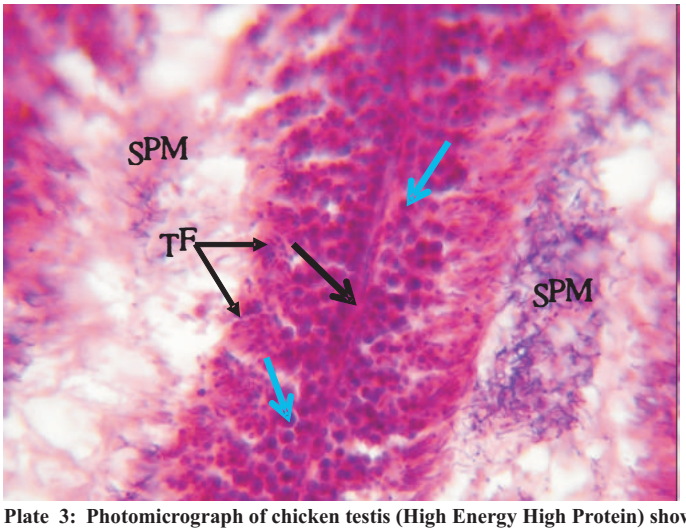

Plate 3: Photomicrograph of chicken testis (High Energy High Protein) showing numerous spermatozoan in the tubular lumen (SPM), thin rim of interstitial connective tissue (black arrow), turf's of spermatids (TF) and spermatogonia lining adjacent to the connective tissue (blue arrows) H&E x400.

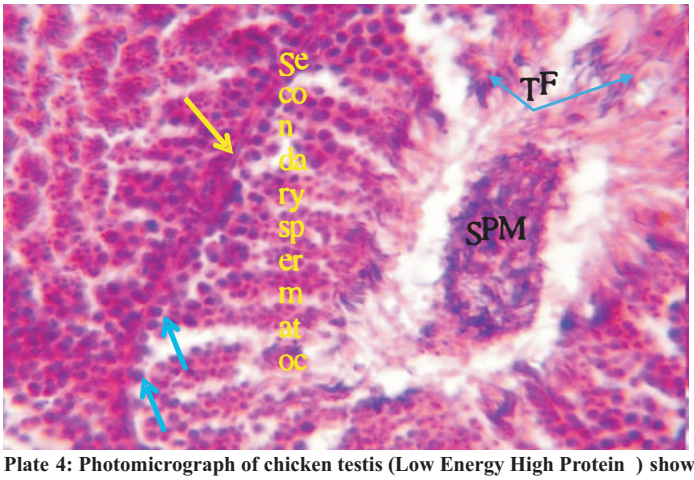

Plate 4: Photomicrograph of chicken testis (Low Energy High Protein) showing aggregation of spermatozoa in the tubular lumen darkly stained (SPM), thin layer of interstitial connective tissue (yellow arrow), zones of secondary spermatocytes, spermatogonia (blue arrows) and turf's of spermatids (TF) H&E x400.

the chickens used.

Histological section of testes of the different treatments in this experiment indicated that HELP combination had some mild effect on spermatogenesis evidenced by the scanty spermatozoa in the tubular lumen. As has been stated before, this might have accounted for the lower fertility observed in the group. It was also observed that this combination gave the highest cocks weight. Semen production and quality have been shown to be affected by male body weight (Alkan *et al.,* 2002; Obi *et al.,* 2013). Bowling *et al.* (2003) observed that the heaviest roosters in the flock had lowest sperm motility, fertility and highest

percentage of sperm with abnormal mitochondria.

Conclusion

It was concluded that FUNAAB – alpha cocks can be fed diets meant for breeding hens without adverse effect on spermatogenesis. However, feeding the LEHP (2400Kcal/kg and 18%CP) diet produced roosters that were overweighed with slightly impaired spermatogenesis.

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