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EFFECT OF VARYING DIETARY ENERGY AND PROTEIN LEVELS ON SEMEN TRAITS OF FUNAAB – ALPHA ROOSTERS

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ABSTRACT

An experiment was conducted to determine the effect of varying dietary protein levels on semen traits of FUNAAB – Alpha cocks. A total of 16 sexually matured cocks reared in individual cages were allotted to four treatment groups in two blocks with each cock serving as replicate in each group. Four experimental diets were formulated as follows; Standard exotic chicken diet (Control), high energy low protein (HELP), high energy high protein (HEHP) and low energy high protein (LEHP). The experimental diets and water were offered *ad libitum* throughout the 34-week experimental period. Semen samples were collected weekly by abdominal massage technique. Semen volume, colour, pH, motility, viability and morphology were determined. Total semen plasma protein, chloride, sodium and potassium were also analysed according to the following methods; Lowry, Volhard and flame photometry respectively. Results of the study showed that dietary treatment influenced ($P<0.001$) semen volume where HEHP was highest (0.90ml). The LEHP group had the highest pH and mass activity. Total motility was highest in the HEHP group (84.95%). LEHP had higher percentages of dead and abnormal sperm cells (11.24 and 3.36% respectively). The mean semen total plasma protein was 14.50, 8.50, 14.00 and 16.00mmol/L for Control, HELP, HEHP and LEHP respectively. Dietary treatment significantly influenced seminal plasma sodium and potassium. No significant difference was observed among treatment means for chloride. It was concluded that specific cock diets with as low as 2600Kcal/kg ME and 13% CP can be fed to FUNAAB – alpha cocks without adverse effects on semen traits.

Keywords: FUNAAB-Alpha roosters, nutrition, sperm quality, seminal plasma chemistry

INTRODUCTION

Rural poultry production is an important agricultural activity of almost all rural communities in Africa. It provides meat and eggs for family consumption and constitutes a reliable source of petty cash. Local chickens also fulfil other functions for which monetary value is difficult to assign. These include their role in pest control, use in traditional ceremonies and festivals, rituals, time clocks, honouring guests, as gifts and in cementing marriages and friendship, alerting owners of the presence of danger and for aesthetic (Nwagu, 2002; Kperegbeyi *et al.*, 2009). In Nigeria, efforts have been geared towards the development of indigenous chicken breeds with improved meat and egg production. One of such breeds is the FUNAAB – alpha chicken developed by the Federal University of Agriculture Abeokuta (Adebambo, 2015).

Nutritional status has been shown to influence the reproductive performance of cocks (Brown, 1994). Circulating nutrients may directly affect the secretion of luteinizing hormone (LH) in the pituitary or indirectly by decreasing signals from median eminence (Martin and Walkden-Brown, 1995). Obi *et al.* (2013) reported that nutrition is important in pre-puberty, puberty and maturity stages of cocks. Cerolini *et al.* (1995) observed that increasing the daily quantity (110, 120, 130

g/bird/d or *ad libitum*) of feed fed to Ross roosters decreased their overall sperm concentration but improved sperm motility, and increased the number of live spermatozoa in the semen. In a study with Dahlem Red birds, Prakash *et al.* (2014) concluded that altering nutrient density in diets by up to 5% higher or lower than NRC recommendation did not influence semen quality parameters of roosters.

Energy and protein are major nutrients influencing reproductive performance of poultry, but optimum dietary energy and protein concentration for FUNAAB -alpha roosters has not been investigated. This study reports the effect of different dietary energy and protein levels on semen quality of the FUNAAB – alpha roosters.

Materials and Methods

A total of sixteen cocks at 32 weeks of age were allotted to four groups with four birds per group serving as replicates and groups allotted to four experimental diets in two blocks(with the position of the cages in the house being the blocking factor). The birds were reared in individual cages for 34 weeks. Four experimental diets were formulated as follows; Standard exotic chicken diet (Control) (Olomu, 2011), high energy low protein (HELP), high energy high protein (HEHP) and low energy high protein (LEHP) as shown in Table 1. The experimental diets and water were offered *ad libitum* throughout the period of the experiment.

Table 1: Ingredients and percentage composition of different energy and protein diets fed to FUNAAB – alpha 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	100.00	100.00	100.00	100.00
Calculated Analysis				
ME (Kcal/Kg)	2600.00	2800.00	2800.00	2400.00
Crude Protein (%)	13.00	10.00	16.00	16.00
†Met + cys	0.53	0.47	0.64	0.60
Lysine	0.61	0.42	0.86	0.82
Calcium (%)	1.06	1.04	1.08	1.09
Phosphorus (%)	0.79	0.70	0.70	0.88

HELP – High energy low protein, HEHP – High energy high protein, LEHP – Low energy High protein
NFE – Nitrogen free extract, ME – Metabolizable energy
† Met+cys – Methionine plus cysteine
*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.

Semen samples were collected weekly by abdominal massage technique (Donoghue and Wishart, 2000) into pre-weighed 2ml Eppendorf tubes® (Eppendorf India)**.** A total of 465semen samples were collected. Semen volume was obtained as the difference between weight of eppendorf tube and sample and weight of empty tube, assuming the density of semen to be 1 g/ml as recommended by the World Health Organization [WHO] (2010).

Semen colour was visually assessed immediately after collection and scored as; 1 = creamy, 2 = creamy-white and 3 = opaque (Peters *et al.,* 2008). A drop of semen was evenly placed on a pH paper (range 6 – 10), after the colour of the impregnated zone became uniform (< 30 seconds) it was compared with the calibrated strip to determine the pH.

Mass activity (gross motility) was determined by placing a drop of raw undiluted semen on a pre-warmed slide and covered with a slip to spread the semen into uniform thickness and viewed under microscope at 100x. Motility was estimated by subjectively assessing the wave pattern and ranked as follows; 4 – rapid swirling, 3 – Slower swirling, 2 – generalized oscillation and 1 – sporadic oscillation (Chenoweth, 2002).

Individual sperm motility was examined at a magnification of 400x. Several fields were examined and an estimate to the nearest 10% of motile sperm was made. The motility determination was carried out by taking into consideration subjective measurements and it was expressed as the percentage of motile sperm with moderate to rapid progressive movement (Cheng *et al.,* 2002)

Percentages live/dead and normal/abnormal spermatozoa were evaluated using the eosin/nigrosin staining procedure (WHO, 2010). One drop of the stain was taken using a pipette and placed at the end of a warm slide and mixed with one drop of semen. A thin smear was made by drawing the edge of a second slide across the mixture. The stained slide was allowed to dry, and then examined using a light microscope at a magnification power of 400x. The live spermatozoa were identified as bright and not stained. Their outlines were clearly defined and their heads were bright and retractile against the backgrounds. The dead spermatozoa stained pink and outlines were not clearly defined. The percentages of live and dead spermatozoa were calculated from 200 spermatozoa which were counted in each slide from different microscopic fields. The morphologically abnormal spermatozoa were also estimated on the same smears by counting 200 spermatozoa in different microscope fields.

Semen concentration was determined with the improved Neubauer haemocytometer (Superior®, Marienfeld, Germany) using the direct cell count method (WHO, 2010). Neat semen was mixed with 0.9% normal saline at the dilution rate of 1:250. The diluted semen was then dropped at two opposite ends of the haemocytometer counting chamber using a micropipette. Counting was carried out at a magnification of 400x. The spermatozoa's head that falls within the sub-divided smaller squares at the four edges and centre of the haemocytometer were counted. The concentration of sperm per volume was determined using the method of Peters *et al.* (2008):

$$C = 50,000 \times N \times D.,$$

Where

C = Concentration of semen per volume (ml),

N = Number of spermatozoa counted,

D = Dilution rate.

Semen samples were collected in labelled plain sample bottles and centrifuged at 3000 rpm for 10 minutes to obtain the seminal plasma which was frozen until required for chemical analysis. Total semen plasma protein was determined using the Lowry method (Lowry *et al.,* 1951). Chloride content of ejaculates was determined by Volhard method (Pearson, 1976). The method uses a back titration with potassium thiocyanate to determine the concentration of chloride ions

in the plasma. Sodium and potassium contents of ejaculates were analysed by flame photometry as described by Kanagasabapathy and Kumari (2000).

Data analysis

Analysis of variance was carried out on data using the General Linear Model of SPSS 20.0 (2011). Where means differed, Duncan multiple range test (DMRT) was used to separate them (Duncan, 1955).

RESULTS

The effects of dietary energy and protein on semen quality are shown in Table 2. Treatment had significant effect on most semen quality parameters measured. Semen volumes were 0.55, 0.75, 0.90 and 0.55ml for Control, HELP, HEHP and LEHP respectively. The HEHP group had significantly (P<0.001) higher volume followed by the HELP, Control and LEHP which had similar values. Semen pH was similar in Control, HELP and HEHP samples but significantly lower (P<0.001) than in LEHP.

Table 2: Effect of Dietary Energy and Protein on Semen Quality of FUNAAB - alpha Cocks

Parameter	Control	HELP	HEHP	LEHP	± SEM
Volume (ml)	0.55^{c}	0.75^{b}	0.90^{a}	0.55^{c}	0.05***
Colour	1	1	1	1	NA
pH	7.13^{b}	7.07^{b}	7.11^{b}	7.29^{a}	0.05***
Mass activity	3.64^{ab}	3.49^{b}	3.77^{a}	3.49^{b}	0.08***
Total motility (%)	80.74^{b}	80.63^{b}	84.95^{a}	79.99^{b}	1.79*
Dead sperm (%)	10.66^{a}	9.92^{a}	5.35^{b}	11.24^{a}	1.84**
Abnormal sperm (%)	1.98^{c}	2.52^{b}	1.70^{c}	3.36^{a}	0.26***
Sperm concentration ($x10^{9}$/ml)	2.66^{a}	1.84^{b}	2.04^{b}	1.76^{b}	0.15***
Sperm concentration/ejaculate ($x10^{9}$/ml)	1.48^{b}	1.38^{b}	1.83^{a}	0.95^{c}	0.14***

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; *: Significant (P<0.05); ** Significant (P<0.01); ***Significant (P<0.001); SEM: standard error of mean; NA – Not analysed

Mass activity was lowest (P<0.001) in the HELP and LEHP fed groups but it did not differ from control which in turn was similar to the HEHP. Individual motility (total motility) was highest (P<0.05) in the HEHP fed group but similar across others treatment groups

Percentage life sperm was significantly (P<0.01) higher in the HEHP group but similar in others. However, percentages dead sperm was significantly (P<0.01) lower in HELHP than in the other groups.

A significant (P<0.001) difference was observed in normal and abnormal sperm percentages across treatment with the LEHP group having significantly lower (P<0.001) proportion of normal sperm, while control and HEHP had similar values. Birds in the HELP group also had significantly lower (P<0.001) value than HEHP but similar to control.

Sperm concentration for control, HELP, HEHP and LEHP were 2.66, 1.84, 2.04 and 1.76 ($\times10^{9}$/ml) respectively. The control diet had significantly (P<0.001) higher concentration

than all the other groups. There was no significant difference (P>0.05) among means of HELP, HEHP and LEHP. Sperm concentration per ejaculate was highest (P<0.001) in the HEHP group, while control and HELP values did not differ and the LEHP group had the lowest concentration.

Table 3 shows the effect of varying dietary energy and protein on semen plasma chemistry. The mean semen total plasma protein was 14.50, 8.50, 14.00 and 16.00mmol/L for control, HELP, HEHP and LEHP respectively. The LEHP group had the highest (P<0.05) value while HELP had the lowest. The control and HEHP values however were not different from those of the HELP and LEHP groups. Mean sodium concentrations were similar in the control, HEHP and LEHP groups. The HELP group had the highest (P<0.05) value but it was not significantly different (P>0.05) from that of HEHP. No significant difference was observed in chloride concentrations across treatments. However, dietary treatment significantly (P<0.01) influenced seminal plasma potassium with the control group having a higher mean compared to HELP, HEHP and LEHP.

Table 3: Effect of Dietary Energy and Protein on Semen Plasma chemistry of FUNAAB - alpha roosters

Parameter	Control	HELP	HEHP	LEHP	± SEM
Total Protein (g/L)	14.50^{ab}	8.50^{b}	14.00^{ab}	16.00^{a}	3.53*
Sodium (mmol/L)	384.50^{b}	675.00^{a}	581.50^{ab}	394.00^{b}	125.11*
Chloride (mmol/L)	94.13	102.78	93.05	109.38	9.95^{NS}
Potassium (mmol/L)	5.25^{a}	3.35^{b}	4.03^{ab}	3.35^{b}	0.78**

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; SEM: standard error of mean; NS: non-significant; *: Significant (P<0.05); **: Significant (P<0.01)

DISCUSSION

The higher semen volume observed on the HELP and HEHP compared to control and LEHP agrees with the observations of Sexton *et al.* (1989) that broiler breeder males fed low energy levels were among the percentage of male that produced lower semen volume. Bramwell *et al.* (1996) also reported a decline in semen volume in broiler breeder cocks fed lower dietary energy levels. Ghonim *et al.* (2010) also reported that drakes fed high level of metabolizable energy or crude protein had significantly higher ejaculate volume. Semen volume and concentration reflect feed utilisation efficiency (Etchu *et al.,* 2013). Birds on the HEHP diet, therefore, had better feed utilization followed by those on HELP as reflected in their semen characteristics. Semen volumes observed in this study are similar to those reported by Peters *et al.* (2008) and Ajayi *et al.* (2011) for normal feathered Nigerian indigenous cocks.

Semen colour was basically creamy white and similar for all the groups. Peters *et al.* (2008) indicated that the further away from creamy white colour the semen of the chicken is the more likely is the presence of contaminations. The pH values observed in the various groups in this study are similar to those reported by Peters *et al.* (2008) for Nigeria indigenous cocks. Similarly, Lake (1981) reported that pH ranges from 7.0 to 7.6, depending on the amount of accessory fluid present. Siudzinska and Lukaszewicz (2008) indicated that chicken sperm can tolerate a pH range of 6.0 to 8.0. Low pH reduces motility, lactic acid production and

oxygen whereas high pH increases metabolic rates *in vitro* (Donoghue and Wishart, 2000).

The HEHP combination supported higher total motility (84.95%). Attia *et al.* (1993) reported that when broiler breeder males were placed on low ME, concern arose because spermatozoa maturation process was altered, motility was also affected, resulting in fewer spermatozoa reaching the sperm storage tubules (SST's). Yahaya (2015) reported higher gross motility in turkey toms fed higher (16 and 20%) as compared to low (12%) crude protein. Tyler and Bekker (2012) however did not observe any difference in sperm motility in Ross broiler breeder males fed three isocaloric diets containing 10.5, 12.6 and 15% crude protein. According to Hafez and Hafez (2008), sperm motility is indicative of its viability and quality. In poultry, it also represents ability to reach the sperm storage tubules (SST's) (Attia *et al.*, 1995).

The observed highest percentage of live sperm in HEHP group and highest percentage of dead sperms in LEHP diet agrees with the report of Ghonim *et al.* (2010) who observed a decrease in percentage dead and abnormal sperm in drakes fed high energy and protein diets. Similarly, Sotirov *et al.* (2002) also observed significant difference in the percentage live sperm of turkeys fed varying levels of crude protein. The high percentage of dead sperm in the LEHP group might have been as a result of the high plasma protein in semen. According to Orlu (2013), there is a 90% chance of semen with high seminal plasma protein to contain high percentage of dead spermatozoa. The LEHP diet recorded more abnormal sperm percentage which is contrary to the observations of Bekker, (2008) who showed no significant influence of dietary protein levels (10.46, 12.61 and 15% CP) on sperm morphology in broiler breeder males. However, Ghonim *et al.* (2010) found that low energy/protein in the diets of Domyati drakes resulted in higher percentage of abnormal sperm. Alkan *et al.* (2002) stated that morphological abnormalities/defects in spermatozoa could decrease the fertilizing ability while Leeson and Summers (2000) stated that the percentage of dead and abnormal spermatozoa in a sample should be less than 10% for optimum fertility.

Sperm concentration was greatest in the control group while higher concentration per ejaculate was supported by HEHP diet. Contrary to what Zhang *et al.* (1999) reported that broiler breeder males fed either 12 or 16% crude protein diet from 21 to 54 weeks did not show any difference in sperm concentration. However, Hocking and Bernard (1997) reported that broiler breeder males fed a 16% crude protein diet had lower sperm concentration as opposed to those fed a 12% crude protein diet. Similarly, Ghonim *et al.* (2010) reported that high energy and crude protein supported higher sperm concentration in male birds. The LEHP group had the lowest sperm concentration per ejaculate. This result supports those reported by Sexton *et al.* (1989) that daily dietary energy intake below the basal level resulted in decreased sperm concentration. The sperm concentration (1.76 to 2.66 x 10^9/ml) observed in this study are similar to the 2.26 x 10^9/ml and 2.24 x 10^9/ml reported by Bah *et al.* (2001) and Machebe and Ezekwe (2004). It is however lower than the 4.05 and 3.45x 10^9/ml reported by Peters *et al.* (2008) for the normal feathered and Alpha Nigerian cocks respectively.

Seminal plasma protein followed a similar pattern with average daily protein intake where HELP had the lowest protein intake and LEHP had the highest. This is an indication of a direct relationship between dietary protein intake and semen plasma protein. The blood plasma constituents are a reflection of seminal plasma contents and vice versa (Ghonim *et al.*, 2010). Bunchasak *et al.* (2005) demonstrated that as crude protein level of the diet rises, blood plasma protein also rises. This may explain the relation between the dietary protein intake and semen plasma level in this experiment. The observed means for seminal protein in this study is within the range of 1.54 to 4.80g/100 mL

reported by Orlu (2013) for the Nigerian indigenous roosters.

The mean semen plasma Na^+ was higher in the groups with high energy irrespective of the protein level. It therefore seems that high energy (not crude protein) in the diet raises the level of sodium in the seminal plasma. Seminal plasma is an important constituent of semen and has a vital role in sperm metabolism, function, survival and motility. Cations such as Na^+, K^+ and Cl^- in the seminal plasma establish osmotic balance and seminal plasma osmolality ultimately plays an important role in sperm cell activation (Alberts *et al.,* 1994; Karaca *et al.,* 2002; Verma *et al.,* 2009). Karaca *et al.* (2002) also revealed that male fertility is impaired by decreasing seminal plasma and intracellular ion concentrations.

CONCLUSION

Specific cock diets with as low as 2600Kcal/kg ME and 13% CP can be fed to FUNAAB – alpha cocks to be used for semen collection and subsequent insemination.

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