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Semen characteristics and sperm morphology of rabbit bucks fed diets containing varying levels of brewers' dried grain

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Target Audience: *Researchers, Animal Reproductive specialist and Farmers.*

Abstract

A study involving 20 adult rabbits was carried out to evaluate the response of feeding brewer's dried grain (BDG) on the semen characteristics. The rabbits were randomly allotted into four treatment groups with five (5) rabbits per treatment and one (1) rabbit as replicate in a completely randomized design (CRD). Treatments 1 (T_1), 2 (T_2), 3 (T_3), and 4 (T_4), were fed diets with 0 %, 20 %, 30 % and 40 level of inclusion of BDG, respectively. Feed and water were served ad libitum. All recommended managerial practices were observed. There were no significant ($P>0.05$) changes in semen characteristics (Volume, concentration, motility, pH etc) across treatments, semen volume was slightly higher in bucks fed BDG diets than those fed the 0% BDG diet. There were slight declines in sperm concentration and motility with increase in the level of dietary BDG. Per cent live sperm was slightly higher in bucks fed 0% BDG diets than in those fed BDG diets. The semen of bucks fed 40% BDG diet had significantly ($P<0.05$) higher per-cent live sperm cells than those fed the other diets. However, % live cells were not significantly ($P>0.05$) different in semen of bucks fed 0 and 30% BDG diet, both of which were significantly ($P>0.05$) higher than in those fed the 10% BDG diet. There were no significant ($P>0.05$) differences in the occurrence both coil and bent tails across treatments, however, they were slightly lower in bucks fed 40% BDG diets compare to those on the other diets. It can be concluded from the findings of the study that, inclusion of BDG in the diets of adult rabbit bucks could be for improved semen volume, concentration and sperm motility.

Keywords: Rabbit buck; semen; brewers' dried grain; reproduction

Description of Problem

In most mammalian species including rabbits, heat stress has deleterious effects on reproductive functions. Exposure of animals to a hot environment causes an increase in body temperature in domestic animals. High ambient temperature also causes a decrease in the length and intensity of estrus by disturbing ovarian function as well as decreasing pregnancy rate after artificial insemination. It also adversely affects the reproductive performance of both sexes (1).

In male rabbits, the testicle is the organ of male reproductive tract responsible for spermatogenesis. The testicular temperature must range from 2 to 8^0C below the body temperature to ensure successful spermatogenesis (2). Upper tolerable temperature for spermatogenesis is 30°C (3). This temperature is maintained by a cooling system comprising the scrotum, pampiniform plexus and muscles (4). Factors associated with sperm quality such as motility, morphology, semen volume, sperm concentration, membrane oxidation status, heat and oxidative stress and other endpoints determine rabbit reproductive physiology (1; 3). Testes are particularly sensitive to heat

stress because they can be influenced by the physiologic abdominal temperature (5). High testicular temperature, either acute or chronic, impairs spermatogenesis and results in a reduction of spermatozoa number associated with a transitional period of partial or complete infertility in several species (6). High ambient temperature was found to affect the total sperm production/total sperm output due to degeneration of germinal epithelium, atrophy of the seminiferous tubules and decrease in the activity of Sertoli cells which in turn affects spermatogenesis (7). During hot period, increased ambient temperature and humidity have been reported to cause an adverse effect on libido, semen production and quality of rabbit bucks due to decreased level of blood plasma testosterone (8; 9).

Seleem *et al*. (10) reported higher sperm motility and live spermatozoa values as well as lower total abnormalities in autumn and winter than summer season due to high environmental temperature. In addition, exposure of adult male rabbits to hyperthermia adversely affects spermatogenesis and decreases testosterone levels (11). Sexual behavior, sperm production and quality vary with health status, environmental conditions and body weight of farm animals (12). Some quality traits have relevant phenotypic correlations with rabbit male performance, such as motility, percentage of abnormal spermatozoa, pH of the ejaculate or the presence of cytoplasmic droplets (13; 14). The lower value of sperm cell concentration ($x10^6$) associated with hot climate could be attributed to decline levels of testosterone and gonadotrophins essential for maintaining the testicular sperm producing potential (7). Increased temperature-humidity index (THI) under high ambient temperature and relative humidity could lead to significantly high percentage of sperm abnormality (15). Therefore, the objective of the study was to evaluate the effect of brewers dried grain on the semen characteristics of rabbits.

Materials and Methods

The study was conducted in the Rabbit Unit of the National Animal Production Research Institute (NAPRI), Shika, Zaria. Shika is geographically situated on latitude 11° 12′42′′N of the equator and longitude 7°33’14” E with an altitude of 691m above sea level (16).

Experimental animals and management

Twenty adult bucks and sourced from the National Animal Production Research Institute/Ahmadu Bello University (NAPRI/ABU), Shika-Zaria were used for the study. The rabbits were grouped into 4 dietary groups and comprising 5 rabbits per treatment group. The rabbits were equally assigned to one of the following levels of BDG (0, 20%, 30% and 40%) weighed and randomly allotted in a completely randomized design. Each group constituted a treatment and each rabbit within a group a replicate. The rabbits were fed 100 g of concentrate daily and given 100 g of hay as supplement three times weekly.

Diet was formulated to meet the nutrients requirement for rabbits (17). They were fed every morning with the experimental diet (concentrate) containing 18-22% crude protein and 2500-2600 Kcal ME/Kg feed. Drinking water was provided daily *ad libitum*. The animals were allowed to adjust to the experimental diet for a period of one week. They were kept in individual metal wire cages measuring 120 × 50 × 60 cm and 75 cm above the ground level. Earthen pots were used as feeders and waterers in each cage. Coccidiostat and anti-stress drugs made of vitamins and electrolytes were administered to the animals at the beginning of the studies. Routine

management operations such as regular cleaning and disinfection of pens, cages, feeders and waterers were carried out regularly throughout the study period.

Proximate Analysis

Samples of BDG were analysed according to the methods described by AOAC (18) at the Biochemical Laboratory, department of Animal Science Ahmadu Bello University, Zaria.

Table 1: Proximate analysis of the BDG

Nutrient (%)	Percentage%
Dry matter	97.33
Crude Protein	97.33
Crude Fibre	16.56
Ether Extract	18.71
Ash content	17.08

Semen Collection and Evaluation

At the end of the experiment, a teaser doe was used and semen was collected using artificial vagina. Volume of ejaculated semen was measured using a 2ml calibrated collecting tube (19), any gel in the ejaculate was discarded. Percentage motility of spermatozoa was estimated by taking one drop of fresh semen from a test tube with a warm Pasteur pipette and placed on a clean warm slide. The drop was covered by a warm cover slip and immediately examined under a warm high power magnification (x400). Percentages of live spermatozoa and differentiation between live and dead spermatozoa were assessed by eosin-nigrosine stain technique. Duplicate smears were made and a total of 200 spermatozoa were counted. Live spermatozoa were not stained while dead spermatozoa were stained according to Dott and Foster (20). Semen pH was measured using chemo craft indicator paper which is calibrated 1-14 with various colours showing the pH. A part of the paper was immersed in the semen, removed and placed on the colour chart: the corresponding colour was recorded as the pH value.

The morphological evaluation of normal and abnormal spermatozoa were determined in the same smear prepared for live/dead ratio observed under x400 objective lens using an olmpus CX21 microscope (model: CX21FS1, Olympus Corporation, Tokyo, japan). The total per hundred spermatozoa was also calculated. Semen was extended 200 fold with physiological saline solution (0.9% NaCl) and a drop of eiosin stain added. Sperm cell concentration was estimated using haemocytometer and Thoma-zeiss cell counter. Examination was made under the high power magnification (x400). Semen quality and morphology was determined according to methods described by (21) and (22).

Feed composition

The feed was milled and mixed at the National Animal Production Research Institute feed mill. The composition of the feed is shown in table 2 below.

Results and Discussion

The semen characteristics of adult rabbit bucks fed diets containing varying levels of BDG are shown in Table 3. Although there were no significant ($P > 0.05$) changes in semen characteristics across treatments, semen volume was slightly higher in bucks fed BDG diets than those fed the 0% BDG diet. There were slight declines in sperm concentration and motility with increase in the level of BDG in the diet. Motility, pH and concentration measured were not significantly different. There was general decline with increase in inclusion of brewers dried grain. This result is agrees with the findings of (23) who did not find a positive effect of vitamin E (40 mg/kg) and selenium (0.7 mg/kg) on sperm motility and

reproductive performance of bucks and (24) who found that using Vitamin C and E did not improve semen qualities. This could be attributed to the low intake of feed observed with increased inclusion levels, thereby missing the ameliorative and antioxidative capacity associated with BDG. Semen volume was lowest in the control treatment; this could be attributed to heat stress.

Table 2: Proximate composition of experimental diets supplemented with brewers drug grains

	T1	T2	T3	T4
Maize	27.60	27.00	27.80	30.00
Maize offal	40.00	34.00	29.20	15.00
BDG	**0.00**	**20.00**	**30.00**	**40.00**
Groundnut cake	27.00	14.00	8.00	10.00
Limestone	0.50	0.50	0.50	0.50
Bonemeal	2.55	2.55	2.55	2.55
Common salt	0.25	0.25	0.25	0.25
Vitamin Premix	0.25	0.25	0.25	0.25
L-Lysine	0.77	0.77	0.77	0.77
DL-Methionine	0.68	0.68	0.68	0.68
Total	100	100	100	100
Calculated analysis				
Crude protein(%)	20.09	18.07	17.27	18.99
ME(kcal/kg diet)	2649	2651	2651	2659
Crude fibre(%)	6.45	7.40	7.76	7.64
Ether extract(%)	4.29	4.65	4.83	5.18
Calcium(%)	0.92	0.95	0.97	1.00
Phosphorus(%)	0.48	0.49	0.49	0.50
Lysine(%)	1.36	1.30	1.27	1.35
Meth. + cysteine (%)	1.14	1.17	1.19	1.28
Feed Cost/25kg(N)	1575.05	1476.30	1436.40	1440.60

ME=Metabolizable Energy, kcal/kg= kilocalorie per kilogram, Meth.= Methionine

Table 4 represent the sperm morphology of adult rabbit bucks fed diets containing varying levels of BDG. Per cent live sperm was slightly higher in bucks fed 0% BDG diets than in those fed the BDG diets. The semen of bucks fed 40% BDG diet had significantly ($P < 0.05$) higher per cent live sperm cells than those fed the other diets. However, % live cells were not significantly ($P>0.05$) different in semen of bucks fed 0 and 30% BDG diet, both of which were significantly ($P > 0.05$) higher than in those fed the 10% BDG diet. There were no significant ($P>0.05$) differences in the occurrence both coil and bent tails across treatments, however, they were slightly lower in bucks fed 40% BDG diets compare to those on the other diets. Supplementation of brewers dried grain improved detached head, free tail and normal cells of the sperm with increased levels of supplementation. This could be attributed to the ability of brewers dried grain which could serve as antioxidant to possibly prevent the excessive production and negative impact of reactive oxygen species in the testes of the bucks. Heat stress under high ambient temperatures has been reported to induce excessive production of oxidants which results in oxidative stress contributing to increased semen morphology in rabbit bucks (25; 26). The findings of this study agree with those of (27; 1).

Table 3: Semen characteristics of adult rabbit bucks supplemented with brewers dried grain

	Level of BDG inclusion					
Parameters	T1	T2	T3	T4	SEM	P-Value
Volume (ml)	0.25	1.05	0.53	1.03	0.38	0.4591
Motility (%)	91.25	86.25	80.00	75.00	5.38	0.2165
pH	6.75	6.75	6.50	6.75	0.26	0.8732
Concentration ($x10^6$)	123.50	123.75	121.25	120.25	16.29	0.1438

SEM=Standard error of mean

Table 4: Sperm morphology of adult rabbit bucks fed diets supplemented with brewers dried grain

	Level of BDG inclusion					
Parameters (%)	T1	T2	T3	T4	SEM	P-Value
Live cells	88.75	77.50	85.00	78.75	5.79	0.4991
Dead cells	11.25	22.50	15.00	21.25	5.79	0.4991
Detached Head	7.00^{ab}	9.00^{a}	4.00^{b}	4.50^{b}	1.05	0.0198
Free Tail	4.00^{ab}	9.00^{a}	1.00^{b}	2.75^{b}	1.48	0.0138
Coil Tail	2.00	2.25	4.00	1.00	1.09	0.3143
Bent Tail	3.75	3.75	5.00	2.25	0.74	0.1277
Normal Cells	82.75^{ab}	76.00^{b}	86.00^{a}	89.50^{a}	2.03	0.0034

Conclusion and Applications

1. It can be concluded from the findings of the study that, 20 % inclusion level of BDG performed better with regards to sperm volume and concentration.
2. Normal cells improved with 30 % and 40 % of inclusion respectively but highest with 40 %.

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