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Semen characteristics of pubertal Yankasa rams fed *Zingiber officinale* supplemented diets

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Abstract

Ginger (Zingiber officinale) is consumed by humans and has been reported to possess medicinal uses. It possesses androgenic property with significant increase on male reproductive parameters. This study assessed the reproductive performance of Yankasa rams fed diets supplemented with different levels of ginger powder. Ginger powder was added at 0, 5, 10, 15 and 20g/kg of the concentrate diet as T1, T2, T3, T4 and T5 respectively. Each treatment had five replicates while semen was collected once from all replicates in the treatments. Rams were fed experimental diets for 70 days. The parameters determined were: mass activity, motility, live:dead ratio, sperm volume, scrotal circumference and length. Mass activity values ranged from 0.67 in T5 to 3.00 in T3. However, there were significant increases in the mass activity of the ejaculate with increase in ginger inclusion up to T3 and subsequent reduction in T4 (1.33) and T5 (0.67). Similarly, scrotal length increased from 14.33cm in T1 to16.33cm in T3, but decreased to 13.33cm in T5. No significant difference was observed in motility, liveability, volume of ejaculate and scrotal circumference, but numerical increases were obtained for motility, live:dead ratio, volume, sperm count and scrotal circumference. It can be concluded that ginger has positive effect on the improvement of semen quality of rams fed ginger powder supplement up to 10g/kg.

Keywords: ginger powder, liveability, semen quality, scrotal length, ejaculate

Introduction

Medicinal plants are of great importance to the health of individuals, communities and animals. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and animal systems. Ginger is one such potential rhizome with a wide range of medicinal effects. It is a well-known plant and is widely used as a spice and medical treatment for certain ailments in traditional medicine (Larsen *et al*., 1999; Mohd-Yusof *et al*., 2002; Tapsell *et al*., 2006; Zhang *et al*., 2009).

Ginger root contains several compounds which have biological activities such as antioxidant and anti-stress properties (Lakshmi and Sudhakar, 2010), antimicrobial and pharmacological effects (Akoachere *et al*., 2002; Rabadah *et al*., 2004; Ali *et al*., 2008). Ginger contains several active compounds including gingerol, shogaols, gingerdiol, and gingerdione (Kikuzaki and Nakatani, 1996; Zhang *et al*., 2009; Zhao *et al*., 2011). Duke et al., (2002) and Kritikar and Basu (2007) reported that ginger possess some aphrodisiac properties. Quaresh et al., (1989) noted that ginger extract significantly increase sperm motility and quality. Consequently, this study was designed to evaluate semen characteristics of pubertal Yankasa rams fed Zingiber officinale supplemented diets.

Materials and Methods

Dried ginger root was purchased from Bode herbs market in Ibadan, Oyo State. All

samples were ground in a laboratory mill to pass through a 1 mm screen. Dry matter (DM) was determined by drying the samples at 105°C and ash was determined by igniting the samples in muffle furnace at 525°C for 8h and nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1995). Crude protein (CP) was calculated as N × 6.25. Ether extract (EE) was determined by extracting the sample with ether (AOAC, 1995). The study was carried out in the sheep unit of Teaching and Research Farm, University of Ibadan, Ibadan. The farm is located on 7^0 27'N and 30 45'E at altitude 200 – 300 m above sea level. In a 70 day study, 25 healthy Yankasa pubertal rams weighing 10.80±0.38 kg were used for the experiment. The rams were given prophylactic treatment of Oxytetracycline L. A. at 1ml/10kg body weight and Ivomectin ® at 1ml/ 25kg body weight respectively. The rams were housed in individual pens with concrete floor and open sided walls; they were offered the experimental diet (Table 1) and water *ad libitum*. The rams were allotted to five dietary treatments in a complete randomized design with each treatment having five animals and each ram stood as a replicate.

Semen collection

Semen was collected on 70th day from the rams using electro-ejaculation (EE) method. The electro-ejaculator was used with a rectal probe of about 22 cm long, 2.5 cm in diameter and with two electrodes. The rectal probe was lubricated and gently inserted into rectum, and orientated so that the electrodes were positioned ventrally. The electro-ejaculator was used in automatic setting, applied for few seconds with 2-seconds rest intervals between stimuli, increasing the voltage stimuli by one volt at a time. However, before the collection, the rectum was washed with 6% sodium chloride solution. The probe was then inserted up to about 12 inches and held in a position of rectal floor. The penis was prolapsed beyond the prepuce, and semen was collected into a graduated collection vial and analyzed immediately at room temperature. The current was alternated with voltage increasing gradually from 0 to 5 volts and returning to zero at every 5 to 10 seconds. The subsequent stimulations were made progressively higher so that at about the fifth stimulus a maximum of 10-15 volts was reached. Erection and ejaculation was obtained. The source of electric current was AC/220-250 volts/single phase/50 cycles.

Semen evaluation

After collection of samples by electro-ejaculator, the volume of each ejaculate was measured in a graduated tube. The proportion of spermatozoa with intact apical ridge was evaluated. After fixation in a buffered 2% glutaraldehyde solution and examined under Differential Interference Contrast microscope at magnification of 400. Total number of spermatozoa per ejaculate was calculated as the product

Table 1: Concentrate composition of the diet fed to the experimental rams.

Ingredient	%
Dry cassava peel	35.00
Palm kernel cake	12.00
Rice husk	8.00
Table salt	3.00
Limestone	2.00

between sperm concentration and volume of the ejaculate. The total abnormal spermatozoa (considering all normal forms in sperm head, intermediate piece and tail) were estimated.

Sperm volume: The volume of the ejaculate was measured with a 5ml graduated cylinder. The sample volume was determined directly in the collection tube by weighing. Thereby, loss of volume associated with transfer from the collection tube to either another tube or a pipette was avoided (Jørgensen *et al.*1997).

Sperm motility: Sperm motility (%) was assessed by the method described by Zemjanis (1977). The evaluation was done with microscope within 2 to 4 minutes of sperm isolation from the caudal epididymis.. A fixed volume of semen (not more than 10 ml) was delivered onto a clean warm glass slide with a few drops of 2.9% sodium citrate and covered with a 22x22 mm cover slip. The preparation was then examined at a magnification of x400 under a light microscope.

Percentage livability: A drop of semen was mixed with 1% eosin and 5% nigrosine in 3% sodium citrate dehydrates solution for the live/dead ratio as described by Wells and Awa (1977). On a clean, warm glass slide, a drop of semen was placed as well as two drops of Wells and Awa stain. The semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was counted and their total percentage estimated (Well and Awa, 1977).

Scrotal Circumference and Scrotal Length: Morphometric measurements of the rams' testes (i.e., scrotal circumference and length) were performed once on the last day of the experiment using a tape measure. The scrotal circumference was measured at the point of the greatest circumference of the scrotum, whereas the scrotal length was measured by obtaining the vertical distance between the ventral abdominal wall and distal poles of the testes.

Statistical analysis

Data obtained were subjected to analysis of variance (SAS, 2000) and where significant difference occurred means were separated using Duncan Multiple range test of the same package.

Results and Discussion

Table 2 shows the proximate composition of the supplemented diet with the ginger inclusions, no significant difference was observed in all the proximate fractions among the different treatments. Table 3 shows the effects of varying inclusion levels of ginger on semen characteristics. There were significant increases in sperm mass activities up to 10g/kg (T3) inclusion level which decline rapidly as the level of ginger inclusion increased in the diet. However, there was a wide range in the value obtained between the control diet (T1) and the other treatments. The ejaculate volume follows the same trend as it increases from 0.13ml in the control diet (T1) to the 10g/kg (T3) level of inclusion and decreases significantly with increasing level of ginger.

The result obtained from semen characteristics for mass activity and ejaculate volume is similar to the result of the experiment by Yates *et al.* (2010) reported an enhancement in the seminal quality of mature goats receiving Tasco at 2% DM compared to controls and as well on male broiler chickens reported by Shannon (2011), wherein there were significant increase in ejaculate volume and sperm

Table 2: Proximate analysis (g/100 g DM) of concentrate with varying levels of Zingiber officinale root.

Parameters (%)	T1(0g/kg)	T2(5g/kg)	T3(10g/kg)	T4(15g/kg)	T5(20g/kg)	SEM
Dry matter	85.05	85.20	85.26	85.37	85.42	0.10
Ether Extract	19.93	21.60	21.73	21.80	21.87	0.52
Crude fibre	20.07	20.98	21.13	21.73	21.93	0.79
Crude protein	11.23	11.47	12.18	12.35	12.94	0.32
Ash	11.00	11.17	10.88	10.63	10.16	0.32
Nitrogen Free Extract	33.63	34.57	34.27	34.13	34.64	0.94

abc= Means on the same column with similar superscript are not significantly (P > 0.05) different

motility with increase in ginger level in broiler feed at 5 and 10kg/ton of feed. This result may be due to the strong antioxidant nature of ginger which either hinder or halt free radicals production.

The scrotal length and circumference were measured in this study, while no statistical significance was observed for the scrotal circumference; significant effect was observed for the scrotal length. Inclusion of ginger at 0g/kg (T1) had the highest value of 16.33cm which declined with increasing level of ginger inclusion. This was similar to the result obtained by Samara *et al* (2014) wherein feeding lambs with diet containing 5% intact *Ulva lactuca* decreased ($P<0.05$) the scrotal circumference and length of both testicles in rams. Meanwhile, these observations may imply that the negative effects of ginger occur at the local testicular level and not at the systemic level. In conclusion, the results obtained from this study showed that ginger had the potentials of improving the reproductive performance of rams fed diets with ginger powder inclusion. However, further studies on the influence of ginger on biophysiological responses of ram needed to be carried out to further affirm the result obtained in this study.

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Table 3: Semen characteristics of rams fed concentrate diets level with varying levels of Zingiber officinale root at varying quantity.

Parameters	0g/kg	5g/kg	10g/kg	15g/kg	20g/kg	SEM
Mass Activity	0.67^{b}	2.00^{ab}	3.00^{a}	2.33^{ab}	1.67^{ab}	0.37
Motility (%)	70.00	76.67	83.33	76.67	73.33	3.75
Live: Dead (%)	91.00	97.00	96.00	97.00	92.00	1.87
Volume (ml)	0.13	0.40	0.43	0.33	0.30	0.09
Scrotal Circumference (mm)	23.67	21.33	23.33	21.00	20.00	0.65
Scrotal Length (mm)	16.33^{a}	15.67^{ab}	14.33^{ab}	14.67^{ab}	13.33^{b}	0.46

abc= Means on the same column with similar superscript are not significantly (P > 0.05) different

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