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Sperm abnormalities and libido assessment of West African dwarf rams fed diets containing *Tetrapleura tetraptera* (*African Porridge*) fruit meal

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Target Audience: *Tetrapleura tetraptera, sperm, abnormalities, Ram*

Abstract

The effect of Tetrapleura tetraptera fruit meal (TTFM) on the sperm abnormalities and libido test of West African Dwarf rams was assessed in a 20 week study. Thirty five (35) West African dwarf rams weighing between 12.80 and 13.20kg were randomly allotted to five dietary treatments in a completely randomized design. The diets formulated: 0% TTFM, 0.5% TTFM, 1.0% TTFM, 1.5% TTFM and 2.0% TTFM as treatments 1, 2, 3, 4 and 5 respectively. Libido assessment was carried out at 0, 4, 8, 12 and 16th weeks of the experiment. Semen was collected twice at the beginning and at the end of the experiment from five replicates in each treatment using electro- ejaculator. The results showed that the libido increased progressively with the inclusion of TTFM which was only significantly different ($p<0.05$) at 16th week. The best libido was observed with rams fed diets containing 1.5 and 2.0% TTFM. The sperm abnormalities were minimal in all parameters except in abnormal head which ranged from 0.4-1.20% with diet 4 exhibited the most abnormal head. It was therefore concluded that the TTFM can be incorporated between 1.5% and 2.0% into diet of rams to improve the sex drive and reduced sperm abnormalities.

Key words: *Semen; libido; fruit meal; abnormal head.*

Description of Problem

Small ruminants are widely distributed and are of great importance as a major source of livelihood of small holder farmers in tropical Africa. However, indications have shown that the productivity of small ruminants in this system is low and there is ample opportunity for improvement (1). Serious constraints in small ruminant production in Africa have been poor nutrition, prevalence of diseases and parasites and reproductive challenges; these cause high mortality amongst kids and lambs, diminishing the benefits of their reproductive performance (2). Variation in the seminal characteristics is known to be affected by many factors (genetic strain, feeding, health status, rearing conditions, season, age and collection frequency) thus contributing to the large variability in the semen traits (3). Presence of phytosterols, a potent precursor and vitamin E contained in *Tetrapleura tetraptera* fruit for the synthesis of sex hormone will improve the semen characteristics of rams.

Materials and Methods

The study was conducted at the small ruminant unit of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The unit is located in the south western part of Nigeria. The area lies within the rain forest ecological zone and fall within longitude and latitude 7^0-27^0N and 3^0-

25^0E respectively and altitude of 220-300m above sea level with the average rainfall of about 1250mm. The temperature and relative humidity ranges from 30-35^0C and 76-84% respectively. Thirty five (35) West African dwarf rams randomly allotted to five dietary treatments in a completely randomized design with 5 replicates chosen from each treatment between 6 and 8months of age and weighing between 12.80 and 13.00kg were used for the experiment.

The fresh *Tetrapleura tetraptera* fruits were purchased from a reputable market in Ibadan, Oyo State Nigeria. This was identified and authenticated at the Herbarium unit of the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo state, Nigeria. The authenticated fruits were rinsed in sterile water and air-dried for two (2) consecutive weeks at room temperature and later milled into powdery form before compounding with other feedstuffs as fruit meal at 0%, 0.5%, 1.0%, 1.5% and 2.0% inclusion levels for treatments 1, 2, 3, 4 and 5 respectively. Each animal was served with *Panicum maximum* grass *ad-libitum* and concentrate diets at 3% body weight twice daily.

Table 1: Gross compositions of concentrate diets containing varying levels of *Tetrapleura tetraptera* **fruit meal for WAD rams**

	Inclusion levels of TTFM (%)				
	0	0.5	1.0	1.5	2.0
Ingredients					
Corn bran	30.00	30.00	30.00	30.00	30.00
Palm kernel cake	25.00	25.00	25.00	25.00	25.00
Rice bran	20.00	20.00	20.00	20.00	20.00
Wheat offal	15.00	15.00	15.00	15.00	15.00
Groundnut cake	5.00	5.00	5.00	5.00	5.00
TTFM	-	+	++	+++	++++
Dicalcium phosphate	3.00	3.00	3.00	3.00	3.00
*Premix	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

TTFM: *Tetrapleura tetraptera* fruit meal

+ (0.5kg TTFM), ++ (1.00kg TTFM), +++ (1.50kg TTFM), ++++ (2.00kg)

*contains Vitamin A (I.U.) 10,000.000; Vitamin D_2 (I.U.) 2,000,000; Vitamin E (I.U) 20,000; Vitamin K (mg) 2,250; Riboflavin (mg) 5000; Pyridoxine (mg) 275; Biotin (mg) 50; Pantothenic acid (mg) 7500; Vitamin B_1 (mg) 175; Vitamin B_{12} (mg) 15.0; Niacin (mg) 27,500; Folic acid (mg) 7500. Choline Chloride (mg) 400; Antioxidant (mg) 125; Fe (g) 20.0; Zn (g) 50.0; Mn (g) 80.0; Cu (g) 5.0g; I (g) 12.0; Co (mg) 200; Se (mg) 200.

Libido was assessed at four weeks interval (0, 4, 8, 12, 16 and 20th week) by reaction time in seconds as described by 4. Before libido assessment, the female animals were induced by injecting them with 0.5ml of prostaglandin PGFα_2 hormone which made the ewes to come on heat after 8 hours of administration. The reaction time within five minutes was rated with the following parameters between first contact with induced ewes: attempt to mount with penis erected. Mounting with no attempt, mounting with sexual desires or servicing and attempt for further servicing were recorded.

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Table 2: Chemical compositions of experimental diet containing varying levels of *Tetrapleura tetraptera* fruit meal

	Inclusion levels of TTFM (%)				
Parameters	0	0.5	1.0	1.5	2.0
Dry matter	81.50	81.40	81.10	80.90	81.55
Crude protein	15.20	15.28	15.34	15.38	15.43
Ether extract	8.40	8.70	8.95	8.96	9.02
Ash	11.00	10.95	10.75	11.02	10.93
Crude fibre	15.89	15.91	16.05	16.10	16.23
Nitrogen free extract	49.51	49.16	48.71	48.54	48.39
Neutral detergent fibre	48.64	52.62	54.69	58.19	60.38
Acid detergent fibre	34.64	36.84	38.93	43.64	47.19
Acid detergent lignin	9.87	11.64	14.62	16.32	17.11
Hemicelluloses	14.00	15.78	15.76	14.55	13.19
Cellulose	24.77	25.20	24.31	27.32	30.08
Tannin	0.32	0.38	0.45	0.56	0.74
Saponin	0.71	0.73	0.78	0.84	0.95
Flavonoid	2.32	2.44	2.67	2.82	3.54
Alkaloid	1.87	1.86	1.90	2.01	2.23
Hydrogen cyanide	0.12	0.15	0.22	0.25	0.26
Sterol	0.76	0.96	1.11	1.36	1.45
Macrominerals (g/kg)					
Calcium	0.84	0.92	1.24	1.68	2.31
Phosphorus	1.12	1.32	1.65	1.97	2.22
Magnesium	2.47	2.54	2.95	3.54	4.01
Potassium	0.74	0.56	0.98	0.79	0.98
Sodium	0.24	0.28	0.28	0.31	0.34
Microminerals(mg/kg)					
Manganese	234.12	242.23	251.23	264.33	267.67
Iron	184.60	177.80	173.30	195.45	205.54
Copper	11.34	8.79	10.33	11.65	10.98
Zinc	55.32	44.76	45.65	51.21	48.87

Libido scoring system for ruminants as used for the experimental animals.

1- Buck shows no interest
2-The sexual interest is shown only once
3-Positive and active sexual interest more than once
4-One mount or mounting with no attempt
5-Two mounts or mount attempt with no service
6-More than two mount with no service
7-One service followed by no further sexual interest
8-One service followed interest include mount and mount attempt
9-Two service followed by sexual interest with mount/mount attempt.
10- The service followed by interest with mount, mount attempt and further service.

According to (4)

Semen was collected at the beginning of the experiment and at the end of the feeding trial from each ram using electro-ejaculation (EE) method. The electro-ejaculator with a rectal probe of about 22cm long, 2.5cm in diameter has two electrodes. The rectal probe was lubricated and gently inserted into rectum, and oriented so that the electrodes are positioned ventrally. The electro-ejaculator was then 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. The penis prolapsed beyond the prepuce, and semen collected into a graduated collection tube. The probe was then inserted up to about 12 inches and held in a position of rectal floor. Alternative current increasing in voltage gradually from 0-5 volts and returning again to zero within 5 to 10s passed. The subsequent stimulation made progressively higher so that at about fifth stimulus a maximum of 10-15 volts is reached. Erection and ejaculation was obtained. The source of electric current was AC/220-250volts/single phase/50 cycles. After collection by electro-ejaculator, the volume of each ejaculate was measured in a graduated tube. The proportion of spermatozoa with an intact apical ridge evaluated. After fixation in a buffered 2% glutaraldehyde solution and was examined under differential interference contrast microscopy at magnification of ×400. The total number of spermatozoa per concentration and volume of the ejaculate were determined. Percentage of abnormal spermatozoa (considering all normal forms in sperm head, intermediate piece and tail) estimated. The sperm colour was determined using a standard colour charts. The volume of the ejaculate was measured with a graduated cylinder. The sample volume can also be determined directly in the collection tube by weighing; assuming 1ml equals 1g. Thereby loss of volume associated with transfer from the collection tube to either another tube or a pipette can be avoided (5).Sperm motility was assessed by the method described by (6) and evaluated microscopically within 2 to 4 min of their isolation from the caudal epididymis and later expressed as percentages. A fixed volume of semen (not more than 10µl) was delivered onto a clean warm glass slide with a few drops of 2.9% sodium citrate and covered with a 22 x 22mm cover slip. The preparation then examined at a magnification of x400 under a light microscope. The percentage liveability was assessed by 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 (7). The morphology was also determined by placing on a clean, warm slide. The smear 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 (7).

Results and Discussion

Semen abnormalities of West African Dwarf rams fed diets containing *Tetrapleura tetraptera* fruit meal

Table 4 reveals the results of semen abnormalities of West African dwarf rams fed diets containing *Tetrapleura tetraptera* fruit meal. There were no significant differences ($p>0.05$) observed at the beginning of the experiment on all parameters of interest. Though not significant, the tailless abnormal sperm values ranged from 0.80-1.20% and 0.40-1.00% both at the initial and end of the experiment respectively. The values of semen abnormalities decreases as the levels of TTFM increased across the treatments. The initial and final values for looped tail recorded were 0.80-1.20 and 0.40-0.80 respectively. The negative variations observed in this study showed that inclusion of TTFM from 0.5-2.0% reduced the semen abnormalities. The significant difference ($p<0.05$) was observed at the abnormal head of the semen. The highest abnormal head (1.20%) was recorded at 0% TTFM while the lowest value (0.4%) for abnormal head was obtained with 2% TTFM. The highest rudimentary tail value (1.00%)

was obtained at treatment four with 0.0% TTFM inclusion level which was not significantly ($p>0.05$) affected by the dietary inclusion of TTFM.

Table 4: Semen Abnormalities of West African Dwarf rams fed diets containing varying levels of *Tetrapleura tetraptera* fruit meal

Parameters	Inclusion levels of TTFM						
	0	0.5	1.0	1.5	2.0	SEM	P-value
Tail-less Head (%)							
Initial	0.80	1.00	1.20	1.20	1.00	0.11	0.34
Final	0.40	0.80	1.00	0.60	0.60	0.17	0.19
Variation	-0.40	-0.20	0.20	-0.60	-0.40	0.19	0.42
Headless Tail (%)							
Initial	0.40	1.40	1.80	1.20	1.00	0.02	0.51
Final	0.80	1.20	0.60	1.20	0.60	0.15	0.30
Variation	0.40	-0.20	-1.20	0.00	-0.40	0.09	0.21
Coiled tail (%)							
Initial	2.20	3.20	2.40	2.80	3.20	0.22	0.13
Final	1.80	2.00	1.80	0.80	1.80	0.22	0.18
Variation	-0.40	-1.20	-0.60	-2.00	-1.40	0.10	0.14
Looped tail (%)							
Initial	0.80	1.00	1.60	0.40	1.20	0.11	0.87
Final	0.80	0.20	1.00	0.20	0.40	0.12	0.84
Variation	0.00	-0.80	-0.60	-0.20	-0.80	0.18	0.41
Looped midpiece (%)							
Initial	1.80	1.80	2.20	2.20	1.80	0.10	0.53
Final	1.40	1.00	1.40	1.20	1.00	0.17	0.81
Variation	-0.40	-0.80	-0.80	-1.00	-0.80	0.35	0.21
Rudimentary Midpiece (%)							
Initial	1.00	1.20	1.00	1.40	1.00	0.13	0.94
Final	1.00	1.20	1.00	0.60	0.80	0.11	0.51
Variation	0.00	0.00	0.00	-0.80	-0.20	0.21	0.32
Rudimentary tail (%)							
Initial	1.40	1.20	0.80	1.00	1.20	0.17	0.86
Final	1.00	0.60	0.60	0.80	0.60	0.12	0.53
Variation	-0.40	-0.60	-0.20	-0.20	0.60	0.22	0.11
Abnormal head (%)							
Initial	1.40	1.20	1.40	1.20	1.10	0.10	0.21
Final	1.20^{a}	0.80^{ab}	1.00^{ab}	0.60^{ab}	0.40^{b}	0.13	0.02
Variation	0.20	-0.40	-0.40	-0.60	-0.70	0.12	0.23

^{a,b,c} Means with different superscripts along the same row are significantly different ($p>0.05$)
TTFM: *Tetraplura tetraptera* fruit meal

Table 5 shows libido assessment of West Africa Dwarf rams fed diet containing *Tetrapleura tetraptera*. The result obtained shows that there were no significant differences ($p>0.05$) observed from week 0 to week 12 in the libido assessment of rams at all levels of inclusion TTFM in the diet with values which ranged from 6.00-8.00 and the highest value for libido was recorded at 2.0% inclusion level has the best value of 8.00 at 12^{th} week of the experiment. Inclusion of *Tetrapleura tetraptera* showed

only significant difference ($p<0.05$) at 16th week. The best libido ranged from 7.60- 7.80 was observed at inclusion levels of 1.5% and 2.0% TTFM respectively. The first mounting and repeated action was showed with rams offered TTFM between 1.5 and 2.0% inclusion levels at 16th and 20th week of the experiment.

Table 5: Libido Assessment of West African Dwarf rams fed with diet containing *Tetrapleura tetraptera* fruit meal

Parameters	Inclusion levels of TTFM (%)						
	0	0.5	1.0	1.5	2.0	SEM	P-value
Week 0	4.33	5.33	4.00	4.67	5.00	0.57	0.83
Week 4	7.00	6.33	5.00	7.33	6.33	0.44	0.29
Week 8	6.00	7.00	6.33	7.00	6.67	0.31	0.51
Week 12	6.60	7.00	7.00	7.00	8.00	0.31	0.90
Week 16	6.20^{b}	7.20^{ab}	7.40^{ab}	7.60^{a}	7.80^{a}	0.15	0.05
Week 20	6.20^{b}	7.00^{ab}	7.40^{a}	7.60^{a}	7.40^{a}	0.17	0.03

^{a,b,c} Means with different superscripts along the same row are significantly different ($p>0.05$)
TTFM: *Tetrapleura tetraptera* fruit meal
SEM: Standard Error of Mean

Spermatozoa are divided into three main segments which are the head, mid-piece and the tail, the head consist of little other than condensed nucleus and overlying acrosome containing acrosin and hyaluronidase (8). The mean values for percentage total sperm abnormalities obtained in the rams of the control group are similar to normal values reported for Yankasa rams by (9) and (10) for West African Dwarf rams. Similar report has been given to cause morphological changes in the germ cells due to gossypol-induced inhibition of the synthesis of sperm-cell histones and other nuclear proteins that stabilize the structure of DNA (11). The finding of our study supports this assertion. There was an increase in percentage total abnormal sperm from week 8 to the end of the experiment in the control group. This agrees with the findings of (12) who reported that the interval between damage to the testis and the appearance of abnormal spermatozoa in the ejaculate is generally between 30 and 60 days, depending upon the site of damage (13). The mean values observed for total sperm abnormalities for the experimental rams were higher than that recommended by (14) as satisfactory for classification of reproductive potential in rams (≤10 %). The inconsistence in the earlier phase of the research could be attributed to many factors which ranges from genetics characteristics of the animal, practices employed and animal adaptability which corroborated with (15) findings that reproductive performance must be considered in light of management and genetics of the breed. The animals were rated based on their sex drive activeness. At 16th week, the significant differences in the treatments ($p<0.05$) in their reproductive performance as treatment 5, 4 and 2 have the highest value of 7.67,7.67 and 7.33 respectively, these significant values obtained can be attributed to increase in the animal adaptability to the feed, environment and management. It was reported that favorable environmental conditions like good feeding and management have the same influence, as selection, and the results attained in this manner are faster and more spectacular (16).

Conclusion and Application

1. Addition of varying levels of *Tetrapleura tetraptera* fruit meal

reduced semen abnormalities except for the abnormal head.

2. The libido improved as the levels of inclusion of *Tetrapleura tetraptera* increased with the length of the experiment

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