Original Article

Veterinary Medicine

Semen Characteristics of West African Dwarf Bucks following the administration of Ethanolic Extract of *Carica papaya* seed

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ABSTRACT [ENGLISH/ANGLAIS]

The effect of ethanolic extract of *Carica papaya* seed on the semen characteristics of eighteen (18) West African Dwarf (WAD) bucks was studied during a period of 14 weeks. The bucks were randomly divided into three groups (A, B and C) of 8 bucks each. 0.1mg/kg, 0.3mg/kg and 0.5mg/kg body weight of the extract was administered daily to group A, B and C bucks, respectively. Data on semen characteristics were collected from each buck before and after the administration of the extract, and analyzed. The impact of the extract on the reproductive ability of the buck led to the reduction in testicular length and circumference, sperm motility and concentration with significant increase in the percentage of some sperm cells with morphological abnormalities, with no recorded side effect. It was also observed that the adverse effect of the extract on the fertilizing ability of these bucks was dose dependent. This study showed that *Carica papaya* seed extract may have the capability to impair fertility in WAD bucks.

Keywords: *Carica papaya* seed, semen, West African dwarf bucks

RÉSUMÉ [FRANÇAIS/FRENCH]

L'effet de l'extrait éthanolique de *Carica papaya* graines sur les caractéristiques du sperme de dix-huit (18) Nain Afrique de l'Ouest (WAD) dollars a été étudiée au cours d'une période de 14 semaines. Les mâles ont été répartis au hasard en trois groupes (A, B et C) de 8 $ chacun. 0.1mg/kg, 0.3mg/kg 0.5mg/kg et le poids corporel de l'extrait a été administré quotidiennement à C mâles groupe A, B, et, respectivement. Les données sur les caractéristiques du sperme ont été recueillies à partir de chaque mâle avant et après l'administration de l'extrait et analysé. L'impact de l'extrait sur la capacité de reproduction de l'argent conduit à la réduction de la longueur des testicules et de la circonférence, la motilité des spermatozoïdes et la concentration avec une augmentation significative du pourcentage de certains spermatozoïdes présentant des anomalies morphologiques, sans effet enregistré de côté. Il a également été observé que l'effet négatif de l'extrait sur la capacité de fertilisation de ces mâles était dépendante de la dose. Cette étude a montré que l'extrait de pépins de papaye Carica peut avoir la capacité de nuire à la fertilité chez les boucs WAD.

Mots-clés: Graines de *Carica papaya*, sperme, mâles nains d'Afrique de l'Ouest

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INTRODUCTION

In recent times, medicinal plants have been found to be of great importance to the health of humans and animals as well as possessing useful active principle for possible beneficial and sometimes adverse effect. Despite the availability of modern medicine, many plants are used in traditional forms as medicine in treating disease conditions with the perception that they produce less adverse effect than synthetic drugs. In West Africa, *Carica papaya*, of the family Caricaceae is one of such plants cultivated for its fruits which are usually eaten raw when ripe or converted to juice, and as a local remedy to some disease conditions as well as a contraceptive in rural areas [1] . Several studies on the *Carica papaya* are focused on laboratory animals [2, 3, 4] thus neglecting livestock's as often times, these animals are fed with various products of the plant. A report, however, has confirmed the contraceptive capability of papaya seed in male lanqur monkeys and its effect on their reproductive ability [5]. Nevertheless, no literature exists on the effect of *Carica papaya* extract on the spermiogram of small ruminants or its trial as a contraceptive in livestock industry. Therefore, this study was designed to evaluate the effect of *Carica papaya* seed extract on the ejaculate characteristics of West African Dwarf (WAD) bucks and its importance on the reproductive abilities of livestock agriculture in this region.

MATERIALS AND METHODS

Experimental Plant Material

The *Carica papaya* fruits were collected from the zoological garden of the University of Nigeria, Nsukka. The fruits were cut open and the seeds were collected. They were dried under the sunlight for few days and later on the laboratory bench to complete its dryness. *Carica papaya* fruits and seed was indentified and authenticated in the biodiversity and conservation unit of the same university.

Extraction of Plant Materials

The dried seeds from *Carica papaya* fruits were grounded into a coarse form using a laboratory miller (Thomas Wiley laboratory mill model 4, USA). 500g of the coarse powder was weighed using an analytical balance mettler, and soaked for 48 hours in one litter of 80% ethanol in a 2.5l Winchester bottle. The bottle was shook intermittently and vigorously after which the content was filtered into a beaker using filter paper (Whatman filter paper) and funnel. The extract was then concentrated into a hot air oven (Gallen Kamp, England) at 40ºC for 72 hours to remove the ethanol and the yield obtained was later stored at 4ºC until use.

Animals and Husbandry

A total of 18 adult (11-13months) West African Dwarf bucks purchased from various goat markets in Enugu State, Nigeria weighing between 6-8kg was used for this study. They were certified to be in good breeding condition by body condition score, scrotal circumference score, and quality of semen, obtained by electro-ejaculation. The animals were kept on the small ruminant unit of the University of Nigeria, Nsukka and allowed to acclimatize for one month prior to the commencement of the study. During this period, they were dewormed (Pantex) and prohylactically treated for any bacteria infection (Pen strep). Daily clinical examination was carried out during this period. The animals were fed on a commercial feed (14% crude protein) supplemented with fresh grass. Clean drinking water was provided ad libitum.

Experimental Design

After one month of acclimatization, they were randomly distributed into 3 groups; A, B and C containing 8 animals each in clean, disinfected separate pens. Group A, B and C received 0.1, 0.3 and 0.5mg/kg body weight of extract, respectively. The extracts were administered intramuscularly on daily basis.

Semen Collection and Evaluation

Baseline parameters were ascertained before the animals were injected with the extract. Semen was collected in all animals using the electro-ejaculation method once a week. All semen samples were collected in graduated transparent plastic tubes attached to a rubber cone, and the semen volume was recorded. Sperm motility, sperm concentration and morphology were evaluated using conventional methods as described [6]. Sperm cell defects were classified into primary (i.e. tapered head, cytoplasmic droplets and aberrant neck-head attachment) and secondary (i.e. detached heads, bent tail, broken tails and coiled tails) forms of sperm abnormalities, and evaluated as described [7]. The testicular length and circumference were measured using a flexible tape rule as described [8].

Statistical Analysis

Data are presented as mean ± SE. Data were analyzed for statistical differences using paired samples T-Test and analysis of variance using Statistical Analysis System software package (SAS). Differences with $p<0.05$ were said to be significant.

RESULTS

The bucks were generally in good bodily and clinical condition throughout the duration of the experiment. The mean weekly values for pre-treatment (Wk 0) and post treatment of the testicular and ejaculate parameters during the experiment are shown in Table 1. Treatment with the extract in the three groups showed a statistical significant difference ($p<0.05$) in the mean testicular length and circumference. The mean testicular length decreased in group A, B and C bucks from 6.67±0.40 to 4.25±0.25, 6.83±0.31 to 3.60±0.40 and 7.37±0.53 to 3.95±0.35, respectively. Also, there was a decrease in mean testicular circumference, and values in the pre-treatment and at 8th week post-treatment in group A, B and C animals were 11.60±0.81 and 8.00±1.00, 13.33±0.36 and 9.20±0.50, and 12.92±0.57 and 8.60±0.00, respectively. There was no statistical significant difference in the volume of semen ($p>0.05$) observed during the study. The colour of all semen samples collected were milky at the start of the experiment but became watery is most semen samples

towards the 6th week post treatment. This was mostly seen in Group C bucks where nearly all semen samples collected became watery at the 5th weeks post treatment.

The mean percentage sperm motility decreased significantly ($p<0.05$) as the weeks post treatment increased. The mean values were highly significant in all groups with values in group C bucks being significantly ($p<0.05$) lower than groups B and A bucks, respectively. Mean percentage sperm motility values at pre-treatment and 8th weeks post treatment in were; group A (80.00±1.83 and 10.00±10.0), group B (78.33±2.11 and 25.00± 5.00) and group C (75.83±2.71 and 0.00±0.00). The mean sperm count decreased significantly ($p<0.05$) as the weeks increased post treatment with group C bucks being the lowest throughout the course of the experiment. The mean value at pre-treatment and 8th week post-treatment for group A, B and C bucks was 355.67±32.96 and 60.50±0.50, 397.83±31.54 and 79.00±2.00, and 293.83±50.20 and 57.00±3.00, respectively. There was a significant increase ($p<0.05$) in the mean percentage dead cells with bucks in group C having mean values significantly higher than the other groups as the weeks increased post-treatment. Mean values pre-treatment and at 8th week post-treatment in group A, B and C was 4.83±1.22 and 66.00±1.00, 6.33±1.23 and 69.50±5.50, and 3.50±2.32 and 89.50±1.50, respectively.

We present the mean weekly changes in some sperm cell abnormalities during the study in table 2. There was no significant change observed with the percentage mean values of tapered head, cytoplasmic droplet and aberrant neck-head attachment. The changes in both detached head and coiled tail during the study were significant ($p<0.05$) in all groups and increased post-treatment. The percentage mean value of the detached head increased from pre-treatment to 8th week post-treatment in group A, B and C was 4 to 14%, 0 to 17% and 2 to 18%, respectively. Also, the percentage coiled tail was 2 to 37%, 4 to 41% and 0 to 35%, respectively. Although, the mean value of bent tail and broken tail fluctuated, there was no significant change during the course of this study.

DISCUSSION

The result from this investigation clearly demonstrate that the administration of *Carica papaya* seed extract had adverse effect on the spermiogram of WAD bucks, and that the higher the dose and continual administration, the greater the effect. This indicates that an increase in the duration and quantity of the seed extract administered or even the consumption of the seed may reduce the reproductive ability of these animals. It also may be the reason why folkloric uses of the plant as a contraceptive or an abortifacient [9, 10]. In the present study, the testicular length and circumference continued decreasing with time in all groups, and that bucks administered with higher doses of the extract showed a greater decline than bucks which was given lower doses. This implies that the effect of the extract on the testis mass was dose dependent unlike result obtained in rats [3] where no change was reported. The semen volume was not statistically significantly affected even though a decrease was observed towards the termination of the study. This may be attributed to the method of collection via the use of the electo-ejaculator which is not very reliable. The ejaculate characteristics are very important to the physiological functions of the spermatozoa and also considered very useful in the evaluation of male animals for breeding soundness [11]. This means that a reduction in the semen characteristics affecting spermatozoa migration and fertilization leads to the rejection of such animal as potential breeders [12]. In this study, there was a significant reduction in the mean percentage sperm motility in all groups, with bucks administered higher dose (group C) being significantly the lowest. These observed reductions in sperm motility in this investigation are similar to earlier reports on male albino rats [4].

The change in semen colour from milky to watery may probably be due to the reduction in the percentage sperm cell count observed in this investigation. This agrees with a similar study where reduced sperm count in rats was reported [3]. The percentage dead cells and cell abnormalities increased progressively with group C mean values being significantly higher than group B and A mean values. This is clearly described by the weekly changes in the sperm morphology, indicating a gradual increase in the percentage sperm cells abnormalities mostly associated with tail abnormalities i.e. coiled tail, bent tail and detached heads i.e. secondary abnormalities which occurs during sperm storage [7]. Therefore, it is possible that the abnormalities may have resulted in the epididymis due to impairment of epididymal function resulting in the disruption of the mid piece and exposure of separate tail fibrils, together will lead to loss of sperm

motility. As a resolution of degenerative lesion, it is likely that *Carica papaya* seed extract may provoke inflammatory reaction in the testicular or epididymal tissue of the bucks, and as such, reduce fertility or remain infertile.

Table 1: Mean weekly values of ejaculate parameter. Numbers of bucks per group = 8

Parameter	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
Testicular length (cm)									
Group A	6.67 ± 0.40	6.42 ± 0.27	5.83 ± 0.33	5.75 ± 0.34	4.36 ± 0.91	4.45 ± 0.25	4.30 ± 0.20	4.25 ± 0.25	4.25 ± 0.25
Group B	6.83 ± 0.31	6.00 ± 0.26	5.73 ± 0.20	5.45 ± 0.24	5.28 ± 0.32	4.65 ± 0.35	4.25 ± 0.25	3.90 ± 0.10	3.60 ± 0.40
Group C	7.37 ± 0.53	6.30 ± 0.45	5.92 ± 0.47	6.10 ± 0.56	5.18 ± 0.28	4.60 ± 0.40	4.15 ± 0.35	4.15 ± 0.35	3.95 ± 0.35
Testicular circum (cm)									
Group A	11.60 ± 0.81	11.30 ± 0.57	10.05 ± 0.69	10.53 ± 0.99	10.35 ± 0.99	8.60 ± 1.10	8.40 ± 1.10	8.00 ± 1.00	8.00 ± 1.00
Group B	13.33 ± 0.36	12.62 ± 0.42	11.92 ± 0.31	11.45 ± 0.49	10.98 ± 0.52	10.25 ± 0.75	9.75 ± 0.75	9.00 ± 0.60	9.20 ± 0.50
Group C	12.92 ± 0.57	11.87 ± 0.61	11.25 ± 0.68	11.55 ± 0.54	11.00 ± 0.68	10.00 ± 0.00	9.85 ± 0.15	9.60 ± 0.00	8.60 ± 0.00
Motility (%)									
Group A	80.00 ± 1.83	75.83 ± 3.00	62.50 ± 4.23	48.75 ± 6.57	42.50 ± 4.79	40.00 ± 5.0	35.00 ± 5.0	16.00 ± 14.0	10.00 ± 10.0
Group B	78.33 ± 2.11	72.50 ± 3.35	65.83 ± 2.39	60.00 ± 3.54	52.50 ± 6.61	50.00 ± 0.00	50.00 ± 5.00	45.00 ± 0.00	25.00 ± 5.00
Group C	75.83 ± 2.71	60.00 ± 2.24	50.83 ± 5.07	35.00 ± 2.89	23.75 ± 3.75	20.00 ± 0.00	10.00 ± 10.00	0.00± 0.00	0.00± 0.00
Sperm count (x10⁶)									
Group A	355.67± 32.96	334.00± 28.40	233.83± 38.31	181.75± 20.28	149.00± 17.62	145.00± 5.00	119.50± 5.00	102.00± 8.00	60.50 ± 0.50
Group B	397.83± 31.54	310.00± 29.66	234.50± 20.97	211.25± 16.72	139.25± 21.77	142.00± 2.00	103.00± 1.00	95.00 ± 2.00	79.00 ± 2.00
Group C	293.83± 50.20	252.83± 38.78	163.67± 21.41	95.50 ± 9.68	96.50 ± 18.86	103.50± 5.50	79.50 ± 13.50	64.00 ± 0.00	57.00 ± 3.00
Dead sperm cells (%)									
Group A	4.83 ± 1.22	8.33 ± 1.02	12.00 ± 2.34	25.00 ± 3.58	34.75 ± 5.39	44.50 ± 5.50	50.00 ± 2.00	60.50 ± 0.50	66.00 ± 1.00
Group B	6.33 ± 1.23	12.83 ± 3.04	28.33 ± 2.91	34.00 ± 5.58	43.75 ± 6.52	42.50 ± 1.23	49.00 ± 7.00	63.00 ± 7.00	69.50 ± 5.50
Group C	3.50 ± 2.32	27.17 ± 3.00	44.83 ± 3.91	57.75 ± 4.44	62.25 ± 2.95	69.00 ± 4.00	78.50 ± 0.50	84.00 ± 1.00	89.50 ± 1.50
Volume (mls)									
Group A	0.16 ± 0.02	0.11 ± 0.03	0.11 ± 0.02	0.11 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.08 ± 0.02	0.06 ± 0.00	0.10 ± 0.06
Group B	0.13 ± 0.01	0.11 ± 0.02	0.10 ± 0.02	0.13 ± 0.03	0.07 ± 0.01	0.06 ± 0.00	0.10 ± 0.04	0.05 ± 0.01	0.04 ± 0.00

Group C	0.16 ± 0.05	0.17± 0.05	0.80 ± 0.02	0.12 ± 0.01	0.13 ± 0.03	0.07 ± 0.03	0.09 ± 0.01	0.08 ± 0.02	0.05 ± 0.01

Table 2: Mean weekly percentage morphological characteristics of ejaculate. Numbers of bucks per group = 8

Weeks	Group	Tapered head (%)	Cytoplasmic droplet (%)	Aberrant neck-head attachment (%)	Detached head (%)	Bent tail (%)	Broken tail (%)	Coiled tail (%)
	A	0	0	0	4	2	1	2
0	B	0	0	1	0	0	0	4
	C	0	0	0	2	0	3	0
	A	0	0	1	30	4	0	2
1	B	0	0	0	4	0	0	3
	C	0	0	0	0	0	0	0
	A	0	0	0	20	7	7	11
2	B	0	1	0	17	6	1	6
	C	0	0	0	0	1	0	0
	A	1	0	0	47	15	5	12
3	B	0	0	2	89	4	1	17
	C	2	6	2	11	10	4	26
	A	2	0	0	0	0	0	32
4	B	0	0	0	21	4	0	7
	C	1	0	2	61	9	5	24
	A	0	0	2	18	2	0	18
5	B	0	0	1	72	1	0	0
	C	0	13	2	61	1	9	34
	A	0	0	0	2	0	0	65
6	B	0	0	0	2	0	0	2
	C	0	2	1	39	3	0	28
	A	0	0	0	0	11	0	31
7	B	0	0	0	10	7	0	38
	C	0	5	0	53	0	11	25
	A	0	0	0	14	22	0	37
8	B	0	0	0	17	13	0	41
	C	0	3	0	18	2	0	35

CONCLUSION

This study shows that ethanolic seed extract of *Carica papaya* administered intramuscularly to WAD bucks (0.1, 0.3 and 0.5mg/kg) for a period of 8 weeks caused significant reduction in the ejaculate characteristics. It also increased the percentage dead cell and some sperm cell abnormalities to a level capable of adversely affecting the fertilizing ability of the bucks with no recorded side effect. However, based on the result in the result in this study, there is a strong indication that more work is needed to determine the effect of *Carica papaya* seed extract on the morphological changes in the gonads of WAD bucks.

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CONFLICT OF INTEREST

No conflict of interests was declared by authors.