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Original Article

Effect of Feeding Roselle Seeds (*Hibiscus sabdariffa* L.) on Some Reproductive Traits of Desert Sheep

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ABSTRACT

Roselle (*Hibiscus Sabdariffa* L.) is a medicinal plant known in Sudan as Karkadeh, which grows successfully in the tropics and sub-tropics, mainly for the production of calyces and epicalyces. This experiment was conducted to evaluate the influence of feeding Roselle seeds on rams' semen characteristics and level of blood estrogen compared with groundnut cake feeding. Nine males of Sudanese desert sheep were allocated to (3) treatments (control group (A), test group (B) and castrated group (C). While the (12) ewes were divided into two groups (control group and test group). Each ram of group A and group B mated with 3 ewes of the experimental females. Group A and C fed with groundnut cake, while group B fed with Roselle seeds .The results showed significant differences ($P<0.05$), in daily feed intake (DFI), average daily gain (ADG) and Feed conversion ratio (FCR) among the experimental groups for rams fed on crushed Roselle. The study showed significant differences ($P<0.05$) among the treatments in semen characteristics (semen volume, motility, sperm morphology and concentration). The study showed significant difference ($P<0.05$) among the treatments in serum estrogen levels. According to the results, Karkadeh (*Hibiscus sabdariffa* L.) seeds had an adverse effect on semen characteristics that might be due to the presence of estrogen in the (*Hibiscus sabdariffa* L.).Based on the current study it is not recommended to use Roselle seeds as an alternative source of protein for reproductive male animals. Roselle seeds could be used for the purpose of fattening of animals.

Keywords: sheep, Roselle seed, reproductive traits, castration.

INTRODUCTION

Sudan is a large country, covering an area of more than 1.850 million square kilometers. The livestock sub-sector in Sudan provides a livelihood for about 14% to 20% of the population; it is concentrated in the Western Sudan States, which have about 39% of the

countries. Livestock in the Sudan satisfies the internal demand and leaves substantial excess for export, which represents about 22% of the country's total exports. Livestock industry has great importance to Sudan economy as it is one of the main sources of food, employment and foreign currency (M.A.R.F., 2008). In recent years, Sudanese sheep namely Sudan desert ecotype, has received great interest as an export commodity to the Arab countries. In the year of 2000 – 2001 for example, sheep exports has contributed with 261.34 million dollars to the national exchange earning at an annual off take rate of 21778 head (M.A.R., 2002).

Sudan Desert sheep tend to breed at certain periods of the year in such a way that lambs are dropped when range fodder is at its best. A few ewes may miss the traditional breeding season and breed in the rainy season to lamb in winter. Some ewes occasionally divert their breeding time to the late of September and October. If allowed to remain in the flock they would lamb during the period of feed and water shortage which lasts from February to May. The off-season ewes are usually identified in early pregnancy and sold for slaughter. To avoid these occurrences, mating is obstructed with the use of a device called a "Kunan". This is a double-looped string fixed around the neck of the scrotum and the neck of the ram's sheath to prevent-the penis from emerging. *Hibiscus Sabdariffa* is a herb belonging to the *malvaceae* family and it is cultivated for leaf, fleshy calyx and seed (Dalziel, 1973)**.** Hibiscus anthocyanin, a group of phenolic natural pigment, present in the dried flower of *Hibiscus sabdariffa* and *Hibiscus rosasinensis*, have been found to have cardio protective hypocholesterolemic, anti-oxidative and hepatoprotective effects in animals, genital tract (Olaleye, 2007)**.** Gaya *et al*., (2009) reported that a preliminary phytochemical screening of the ethanolic seed extract of *Hibiscus sabdariffa* revealed the presence of alkaloids, saponins, tannins, anthraquinones, steroids, cardiac glycosides, flavonoids and phlobatanins. They reported that the lactogenic effect of ethanolic seed extract of *Hibiscus sabdariffa* was investigated by administering extract and metoclopramide in albino rats. Okasha *et al*., (2008) reported phytochemical alkaloids, cardiac glycoside, deoxy sugar, flavonoids, steroidal ring and tannin concentration were moderate, moderately low, high and low respectively when they studied the effect of aqueous *Hibiscus sabdariffa* seed extract on serum prolactin level of lactating female albino rats.

Hibiscus sabdariffa locally known as karkadeh grows successfully as a cash-crop in western Sudan appears to have a great food value and pharmaceutical potential not fully exploited (Karamall's personal communications). Sudan is known to be the world's major supplier of Karkadeh. The Roselle seeds, which are the subject matter of interest in this study, are just a by-product of the crop and its total production is increasing steadily, as a result of increased international demand.

The Roselle seeds have a good potentiality as a new source of vegetable oil and protein (AL–Wandawi *et al.,* 1984). The objective of this study to evaluate the influence of feeding Roselle seeds compared with groundnut cake on sheep's semen characteristic and level of blood estrogen.

MATERIALS AND METHODS

Management of experimental animals

Nine rams of Sudan Desert sheep (ecotype Shugur) at age of 7-8 months, with average body weight of 41.9 kg , and Twelve ewes of Sudan Desert sheep, ecotype Shugur, within age range of 2-3 years with average body weight of 30.13 kg were purchased from Maatoug area, Gezira State – central Sudan. Animals were vaccinated against anthrax and hemorrhagic septicemia, injected with antibiotic as prophylactic measures, dipping in Gamatox solution against external parasite and drenched with broad-spectrum antihelmntic against internal parasite. All animals were allowed to have adaptation period for two weeks, during which they were fed complementary feed (60% concentrate and 40% roughages).The experimental 9 male animals of Sudanese desert sheep were divided into (3) groups each was allocated to one

of the treatments (control group, test group and castrated group), while the (12) ewes, were divided into two groups (control group and test group).

Housing

The experimental sheep were lodged in a semi opened house 7×9 meters enclosed with corrugated zinc set over a half – meter brick wall. The roof was made of corrugated metal sheets, sloping from the middle (3m height) to the sides (2.5m) and supported with metal pipes.The floor was made of concrete with reasonable inclination for drainage. House was partitioned internally to 10 pens (1.5×2 m.). Each pen had a separate door and adequately equipped with watering and feeding facilities.

Experimental diets

Two experimental rations were formulated as complete formula feed, utilizing Roselle crushed whole seed and groundnut meal as a protein sources (Table 1). The experimental diets were labeled A (control) and B (contained Roselle seeds). Ingredients used to formulate the rations were based on sorghum grain, wheat bran, groundnut hulls, molasses and urea. Mixing of the experimental rations was done manually after weighing the recipe. The experimental diets were formulated iso-energetic and iso-nitrogenous to meet or exceed the nutritional requirements of sheeps. The dry small quantity ingredients were mixed first then finally with the molasses with urea. Table 2 shows the chemical composition of the experimental diets. The experimental feeding extended for 10 months.

Table 1- Experimental rations composition (% fresh basis)

Ingredients%	Rations	
	A	B
Roselle seed meal	00.00	30.00
Groundnut meal	20.00	00.00
Dura grain	40.00	30.00
Wheat bran	15.00	10.00
Molasses	10.00	15.00
Groundnut hulls	10.67	10.20
Urea	00.33	00.80
Oyster shell	02.00	02.00
Nacl	02.00	02.00

Table 2- Chemical composition (% dry matter basis) of experimental rations.

Item	Rations	
	A	B
Dry matter	91.24	92.52
Crude protein	23.26	22.33
Crude fiber	20.74	22.88
Ether extract	5.68	7.30
Nitrogen-free extract	20.44	16.77
Ash	10.32	12.50
Energy (MJ/Kg DM)	10.80	10.74

Data collection

Performance

Feed intake (kg/group) was recorded on daily basis by difference between feed offers and residues. Weekly body weights of individual animals were recorded to the nearest 0.5 kg once weekly at 8:00 a.m. before feeding. Feed conversion ratio was calculated weekly as: feed intake/weight gain.

Blood serum

Blood samples were collected from each animal. For male blood, samples were collected biweekly for two months at four occasions and for female, samples were collected at 14 days interval for 6 months (period 1, period 2 and period 3). The blood samples were collected via jugular vein using sterilized 5ml syringes into heparinized tubes for serum separation.

Estrogen determination

Estrogen level of experimental animal serum was determined by using estrogen kit according to Abraham (1975). One ml of reagent with 100 pg/ml four assays were set up simultaneously and incubated for varying periods at 37ºC and then reading the level of estrogen in the tubes.

Semen characteristics

Three rams were selected and fed till reached the age of maturity and trained for semen collection. The volume of the ejaculate was read directly from a graduated collection tube. Individual forward motility was estimated by examining one drop of semen, diluted in 3.8% sodium citrate solution, under a microscope (10×40).

The number of spermatozoa per cubic millimeter was counted using a haemocytometer. A 0.05 ml of semen was drawn into the pipette. A small bubble of air was drawn into the tube. The tube was filled with 3% normal saline to reach 1.01 ml. Then, the dilution pipette was agitated gently for one minute. A haemocytometer was prepared by placing cover slip on ruled filed and a drop of diluted semen was allowed to run under the glass cover and sperm cells were left to settle. The counting was made under (40x) magnification.

Five large squares each one containing sixteen smaller squares was counted (corner and middle chamber). Dead spermatozoa was determined by examining 200 spermatozoa in an eosin nigrosine smear and categorizing them into coloured (dead) and non-coloured (alive) cells under a microscope at high magnification (10×100). The proportion of morphologically abnormal spermatozoa was also determined by examining 200 spermatozoa in an eosin-nigrosine smear under the same magnification.

Testes and scrotum morphometric

Testicular measurements (scrotal circumference, testicular diameter, and testicular weight) were measured as described in the companion paper (Toe *et al*., 2000).

Chemical analysis

The experimental rations were chemically analyzed according to A.O.A.C (1984). Extracted Roselle seed oil was analyzed to assess phytoestrogen, while the blood serum for level estrogen was determined by using estrogen kit according to Abraham (1975).

Statistical analysis

The data were statistically analyzed analyses of variance by using MSTAT program. T-test has been used for semen characteristics for the two dietary treatments. Also, Duncan multiple range tests was used to detect difference between means (Steel and Torrie, 1980)

RESULTS AND DISCUSSION

Feedlot performance of rams

The feedlot performance results of the experimental rams were shown in (Tables 3**).** The Initial body weight and final body weight showed no significant differences (P>0.05) among the treatment groups (A control, B Roselle seed 30% and C castrated). Group B had the

highest value of daily weight gain and group A and C had the lowest values (P>0.05). The high nutritive value of Roselle seed meal may be due to estrogenic compounds or factors are found generally in malvacaes (*Hibiscus Sabdariffa*). Beshir (1996), Hassan (2005) and Darran (2007) reported a similar trend that agreed with the present results, as they found that the rate of weight gain increased with increased level of the Roselle seed meal, which made subordination of Roselle seed content of estrogenic compounds. The plane of nutrition and the nutritive value of ingredients are the major factors affecting the daily feed intake. The ruminants eat until they reach satisfy, while monogastrics eat to satisfy their energy requirements. Thus as the energy content of the ration increased, the amount required to satisfy a certain nutritional requirements decreased. In the experiment the daily feed intake showed a significant difference (P≤0.05) among the treatments. In this study, Lambs fed ration B (Roselle seed) had the less value of daily feed intake.

These results agreed with Mohammed and Idris (1991), who revealed that the feed consumption in poultry decreased as the level of Roselle seed meal increased, and they attributed that to the acid taste, and to high fat content of Roselle seed meal, while disagree with Salihand Abdelwhab (1990) and Bakheit (1993), who reported that the feed intake in poultry increased as the level of Roselle seed meal increased.Feed conversion ratio (FCR) is an important factor reflecting the nutritive value of the feed and the efficiency of utilization of the feed.

The feed conversion ratio in this study showed no significant differences (P>0.05) among the different treatment groups. The best recordof (FCR) was reported with group B (9.24). Roselle seed as protein source for feeding ruminants showed a similar trend of FCR that increased as the level of Roselle seed meal increased (Beshir, 1996; Suliman, 2004; Hassan, 2005; Darran, 2007).

Table 3- The effect of castration treatment and inclusion of Roselle seed meal replacing partially the dietary groundnut meal on feedlot performance of the rams

Items	Groups			±S.E	Treatment effect
	A Control	B (R.S)30 %	C Castrated		
Initial body weight (kg)	42.65	41.35	42.00	0.40	N.S
Final body weight (kg)	53.81	54.07	52.47	0.40	N.S
Total weight gain (kg)	11.22[b]	13.11[a]	10.81[b]	0.32	*
Daily weight gain (kg)/day	0.19[b]	0.22[a]	00.19[b]	0.0001	*
Daily feed intake (kg)/head/day	02.13[a]	02.02[b]	02.14 [a]	0.01	*
Feed conversion ratio	11.20	09.24	11.21	0.33	N.S

[a, b]Means in a row with differing letters differ significantly (P < 0.05); N.S. not significant. * = P < 0.05.

Testicles and scrotum measurements of experimental rams

Testicles measurements include scrotum circumference, length and weight are shown in (Table 4)**.**The study showed significant differences (P>0.05) among the treatments for final scrotum circumference and testicular final length and weight. Rams fed ration A had the highest values, while the other treatments had the lowest values.

There were no significant differences (P>0.05) between rams fed Roselle seed meal in their diet and castrated rams group. The lower values of testicles measurements of group B might be attributed to the phytoestrogenic factors of Roselle seed. Kurzer and Xu (1997) reported that phytoestrogens had estrogenic compounds found in plants which affect the central nervous system, inducing estrus and stimulating the growth of the genital tract of animals.

Adams, (1995) reported that in Australia and New Zealand sheep grazing on estrogenic clover pasture showed symptoms of (clover disease) a fertility disorder due to changes in the genital tract. However, animals in treatment C had testicle atrophy as a result of castration.

Table 4-. The effect of castration treatment and inclusion of Roselle seed meal replacing partially the dietary groundnut meal on scrutum and testicular measurements

Items	Groups			Treatment effect
	A Control	B (R.S)30 %	C Castrated	
Scrotum circumference (cm)	29.33±0.67[a]	26.33±0.33[b]	20.0±0.00[b]	*
Testicular final length (cm)	14.67±0.33[a]	11.78±0.39[b]	9.06±0.05[b]	*
Testicular weight (g)	350±28.87[a]	216±16.67[b]	166±16.67[b]	*

[a, b]Means in a row with differing letters differ significantly (P < 0.05); N.S. not significant. * = P < 0.05.

Semen evaluation

Semen characteristics of the experimental animals were shown in (Table 5)**.** According to Bearden *et al.*, (2004) semen characteristics of the normal spermatozoa are composed of a head and a tail that is divided into mid-piece, main-piece and end piece**.** The study showed highly significant differences (P≤0.01) among the dietary treatments for semen volume, mass motility, abnormal morphology and semen concentration. Treatment A rams had the best values of the good semen characteristics, whereas treatment B rams had the lowest values of the good semen characteristics.

Table 5- the effect of inclusion of dietary Roselle seed meal replacing the groundnut meal on semen of rams

Items	Groups		Treatment effect
	A Control	B (R.S)30 %	
Semen volume (ml)	1.5±0.0[a]	0.89±0.05[b]	**
Mass motility score	4.33±0.33[a]	3.0±0.00[b]	**
Individual motility score	3.67±0.33[a]	3.0 ±0.00[b]	**
Normal morphology (%)	87.0±1.73[a]	72.0 ±1.45[b]	**
Abnormal morphology (%)	13.33±1.76[b]	27.33±1.45[a]	**
Dead sperm (%)	12.33±1.45[b]	21.67±0.88[a]	**
Concentration/ml×10^9	4.06±0.18[a]	3.28±0.9[b]	**

[a, b]Means in a row with differing letters differ significantly (P < 0.01);
N.S: not significant. * *= P < 0.01.

These results recorded may be due to feeding the rams with Roselle seeds meal which contain estrogenic compounds. The average volume of ram semen ejaculate when collected by artificial vagina was about 1.0 ml depending on age and condition of the animals, frequency of collection and skill of operator. Accurate determination of semen concentration is very important for the determination of the dilution rate (Evans and Maxwell, 1987)**.**These results agreed with Darran (2007) who studied the effect of feeding Roselle seed meal on semen characteristic of Sudanese desert rams and Orisakwe *et al.*, (2004) who studied the effect of sub chronic administration of aqueous extract of *Hibiscus Sabdariffa* calyx on rat testes activity.

Estrogen levels in experimental ram's serum

Table 6 showed the estrogen levels in experimental animals**.** The study showed significant differences (P≤0.05) among the treatments for estrogen level in experimental rams serum. The study showed that the level of estrogen concentration increased for those rams of fed Roselle seed meal. The present study findings are in line with the results reported by (Kurzer and Xu, 1997; Adams, 1995; Darran, 2007). They reported that Roselle seed had estrogenic effect when fed to the small ruminants (rams). On the other hand, the study showed no significant differences (P>0.05) among castrated rams and the others fed the control diet.

Table (6). The effect of castration treatment and inclusion of Roselle seed meal replacing partially the dietary groundnut meal on estrogen level in ram blood serum

Items	Groups			Treatment effect
	A Control	B (R.S)30 %	C Castrated	
Level of estrogen with an initial weight.(pg/ml)[1]	12.24±0.13	12.51±0.20	12.09±0.16	N.S
Level of estrogen after two weeks .(pg/ml)	14.22±0.56[b]	17.83±0.91[a]	11.00±0.68[b]	*
Level of estrogen after four weeks.(pg/ml)	17.04±1.73[b]	26.72±1.56[a]	18.13±1.28[b]	*
Level of estrogen after six weeks .(pg/ml)	19.27±0.64[b]	28.77±0.44[a]	17.42±0.53[b]	*

[1]Pg/ml: Pico mol gram/ml[a, b],Means in a row with differing letters differ significantly (P < 0.05); N.S. not significant. *= P < 0.05.

CONCLUSION

Feeding males on Roselle seeds affected the secretion of semen volume and concentration and consequently decreased semen ejaculation. However, males fed on groundnut cake gave good volume, concentration and mass motility (the good semen characteristics) .Therefore, Roselle seed meal is very convenient for feeding castrated males for fatting because it contains high percentage of factors could enhance estrogen which promotes the weight gain and it could be good alternative protein source in sheep diet.

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