Research Article

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Using some seminal characteristics to determine the age at sexual maturity in Sudan Nilotic kids

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Age at sexual maturity in pubertal Sudan Nilotic bucks was investigated by examining some of their seminal physical characteristics and contents of zinc and nitric oxide. 13 bucklings, at 4 months of age, were divided into sorghum meshed (SMD) and molasses pelleted (MPD) diets groups and were exposed weekly to a teaser female. Puberty was recorded at first ejaculate into an artificial vagina and sexual maturity was confirmed when the quality of semen became similar to that of fertile adult bucks. Analysis of covariance (season of birth is the covariate) revealed that feeding treatment had no effect (P>0.05) on age and weight at puberty nor on age at sexual maturity. Seminal nitrate/nitrite and zinc contents were similar in both feeding groups. Reaction time decreased and semen volume increased, but insignificantly, from week 1 after puberty until week 7 when they became comparable to that in the control adult bucks. Sperm mass activity and individual motility increased significantly from week 1 after puberty through week 7. Seminal nitrite/nitrate and zinc levels were not affected (P<0.05) by week of semen collection. Semen volume, sperms mass activity and seminal nitrate levels correlated positively (P<0.05) with each others. It is concluded that Nilotic bucklings attained puberty by about six month of age, but sexual maturity in seven weeks later. There were encouraging highlights on use of seminal biochemical contents as zinc and NO levels as indicators for sexual maturity of bucks, however further studies with larger sample size is needed.

Key words: Seminal nitric oxide and zinc, sexual maturity, Nilotic bucklings

Puberty represents the time at which reproduction first become possible and it is characterized by ovulation in females and ejaculation in males (Lawrence and Fowler, 1997). It is important to differentiate this state from sexual maturity which is the state reached when the animal is capable to express its full reproductive power. Hunter (1980) stated that puberty proceeded adulthood, and it is reached at an age which varies considerably with breed and also among individuals within a breed.

Knowledge of biochemical characteristics of the pubertal buck's semen will be a useful tool in early selection of sires for planned breeding programmes (Butswat and Zaharaddeen, 1988). Many studies have been designed to elucidate a possible relationship between zinc seminal plasma concentration and infertility. Elzanaty *et al.* (2002) noted that seminal levels of zinc and nitrate/nitrite are prostatic markers and that of fructose is a vesicle marker. Fuse *et al*. (1999) stated that there was a positive correlation of zinc concentration with sperm concentration and motility. Donnelly *et al*. (1997) stated that Nitric oxide (NO) is produced directly by spermatozoa. They also noted that endogenous NO appears to have an important role in the maintenance of sperm motility and may be useful as an indicator of fertility with numerous clinical applications in the field of assisted reproduction technologies.

Nutrition is the most important environmental factor that affects reproductive activity in small ruminants. According to Jurgens and Bregendahl (2007) the most appropriate livestock feeding system should be advantageous in ease of access to the feed, ease of cleaning and minimum feed waste. They added that intensive livestock feeding can be standard when concentrate and roughage portions

were offered separately or complete when the two portions were offered in one mixture. The advantages for complete diets appear to be simplicity of management and full mechanization, coupled with economy of feeding space, safety and flexibility for inclusion of a wide range of ingredients (Owen, 1984). Disadvantages include high capital cost and risks of mechanical breakdown. Adam *et al.* (2010) found that feeding complete molasses-based diet to Nilotic bucks resulted in marked feeding cost reduction when compared with sorghum-based diets.

The aim of this study was to examine the age at sexual maturity using some of seminal physical and biochemical characteristics in pubertal Nilotic bucks reared on standard meshed sorghum-based (SMD) or complete pelleted molasses-based (MPD) diets.

MATERIALS AND METHODS

A comprehensive research study was conducted at Juba University farm, 15 km north of Khartoum centre, to evaluate the reproductive and productive potential of Nilotic goats. The parent flock was brought from the Upper Nile State in southern Sudan, consigned to Khartoum and raised intensively under two feeding systems. The first system was composed of two separate portions. The concentrate portion was a sorghum meshed diet (SMD). To this concentrate portion, sorghum straw was offered separately as a roughage portion. The second feeding system was a complete molasses pelleted diet (MPD). Under this system, animals were offered their feed as one complete diet. The concentrate portion of SMD feeding system (24.8% CP and 11.4 MJ ME/kg DM) had crushed sorghum grains (32%) and groundnut cake (36) as the main sources of energy and protein, respectively; wheat bran (29%), salt (1%) and limestone (2%) were also added. Sorghum straw (4.14% CP and 6.22 MJ ME/kg DM) was the roughage portion of this system. On the other hand, the MPD feeding system (15.3% CP and 9.14 MJ ME/kg DM) composed of urea (1.5%) and molasses (35%) as the main sources of protein and energy, respectively, crushed sorghum grain (20%), wheat bran (15%), salt (1%) and limestone (2%) were also added. Bagasses (15%) were incorporated in this diet to increase its fibre content.

In this study, 13 bucklings, at the age of 4 month, from the first crop of the parent Nilotic stock were selected. These animals were divided into two groups according to their dams' feeding systems as described above (5 and 8 animals for SMD and MPD feeding groups, respectively). The season of birth of bucklings was recorded (dry summer: March to June; wet summer: July to October and winter from November to February). The bucklings were exposed weekly to a teaser female. The age of first ejaculate was recorded as the age of puberty. Sexual maturity stage was reached when the characteristic of the collected semen became similar to that of their parents (3 fertile adult bucks for each feeding system). Semen was collected weekly (for 7 weeks) using standard artificial vagina (AV) for small ruminants (IMVO40). Libido was measured as the reaction time represented by the time elapsed from presenting the buck to a female until ejaculation was accomplished. All procedures of semen collection and evaluation were carried out according to the protocol described by Evans and Maxwell (1987).

Seminal plasma was assayed for assessment of nitrite/nitrate and zinc concentrations. The semen samples were centrifuged at 1500 *g* for 5 minutes, the seminal plasma aspirated and stored at –20°C until bioassay. Nitrate and nitrite were produced by steam distillation method as described by Ryan and Estefan (2003). Zinc content was determined by atomic absorption spectrophotometer apparatus. In brief, seminal plasma was diluted 1:100 v/v with distilled water, then 0.2 ml of diluted seminal plasma or control sample was mixed with 1.8 ml of distilled water. The absorbance of each specimen was measured in an atomic absorption spectrometer (Hitachi Model Z8000, Hitachi ltd., Tokyo, Japan). Seminal zinc concentration was determined according to a standard calibration curve obtained from zinc standard solutions (Wako Pure Chemical Industries, Ltd., Japan). The intra- and inter assay coefficients of variation of this assay were 3.0% and 4.0%, respectively.

Taking the season of birth as a covariate, analysis of covariance (StatSoft, 2001) was used to examine the effect of feeding protocol (SMD vs. MPD) and the week of semen collection on the age and weight at puberty; age and weight at sexual maturity and on some physical and biochemical characteristics of semen of the pubertal Nilotic bucklings.

RESULTS

The feeding system had no significant effect (P>0.05) neither on age and weight at puberty nor on age at sexual maturity (Table 1). The MPD group had a significantly (P<0.05) heavier weight at age of sexual maturity than that of SMD group. Reaction time and most of the semen characteristics were similar for both groups. The volume of the ejaculate and individual motility were significantly (P<0.05) higher in the MPD group. The concentrations of nitrate/nitrite and zinc in seminal plasma were similar in both feeding systems.

The reaction time decreased, but insignificantly (P>0.05), from week 1 after puberty until week 7 when it became comparable to that of the control adult bucks (Table 2). The opposite trend was observed for semen volume which increased, but insignificantly (P>0.05), with time. Spermatozoa mass activity and individual motility increased significantly (P<0.05) from week 1 after puberty through week 7. Nevertheless, these motility traits never reached their corresponding values in the control adult bucks. Seminal nitrite/nitrate and zinc levels were not affected significantly (P<0.05) by the week of semen collection.

The correlation coefficients matrix in Table 3 revealed that semen volume, sperms mass activity and seminal nitrate levels correlated positively (P<0.05) with each others. Mass activity also has positive significant correlations with sperm individual motility and nitrite seminal level. Nitrite and nitrate in the semen also correlated (P<0.05) positively with each other. The other traits did not show significant (P>0.05) correlations with each others.

DISCUSSION

In the present study, kids attained puberty at 173 ± 13.4 days of age and 10.9 ± 1.11 kg body weight, but sexual maturity at the age of 224 ± 7.42 and body weight of 12.2 ± 1.54 kg when the characteristics of the examined seminal traits became comparable to those in the adults (control). The age and live body weight at sexual maturity of this study is within the range reported by Butswat and Zaharaddeen (1988) for Red Sokoto and Kano Brown bucks of Nigeria. Devendra and Mcleroy (1982) observed that puberty in male Boer goats in South Africa was reached in 157.5 days old and the count of morphologically normal sperms rose from 36.5 - 89.8% at the 8^{th} week.

Semen evaluation is used to predict the breeding value of a sire used in natural service or artificial insemination (Evans and Maxwell, 1987). The semen volume of pubertal Nilotic bucks was slightly lower than the range of 0.5 to 1.0 ml, reported by Devendra and Burns (1983) as mean volume ejaculate in tropic goat breeds. Mass activity of the semen of the pubertal bucks was not different in the two groups. Although, the MPD group ejaculated significantly more volume with higher individual motility than their SMD counterpart, yet the semen characteristics in both feeding groups fall within the normal range for good quality semen according to Hafez (1980) and Devendra and Burns (1983). Therefore, the sorghum-based feeds can be replaced by the molasses-based diet without affecting the normal growth to maturity in pubertal Nilotic bucks.

No differences were observed between the two feeding groups in zinc level. Moreover, the contents of this element did not change significantly with time between puberty and sexual maturity. In this study, zinc concentration showed an increase with age, although insignificantly, probably due to the small number of observations in this study. This was consistent with the findings of Massnyi *et al.* (2003) who reported that zinc concentration is high in the adult testis. Zinc has been reported to influence the process of spermatogenesis (Wong *et al.*, 2002) and controls sperm motility (Wroblewski *et al*., 2003). This explained the correlation of zinc content with sperms mass activity and individual motility, however it is insignificant.

Nitric Oxide (NO) is a labile and diffusible molecule which forms stable metabolites (nitrite/nitrate) detectable in many biological fluids. Therefore, Nitrite and nitrate (NO_2^-/NO_3^-) concentrations of seminal plasma were determined as measure of nitric oxide (NO) level. In the present study, no difference was observed between the two diet treatments in nitrate/nitrite levels. Again the levels of these metabolites did not change significantly with time between puberty and sexual maturity ages. In this study, the concentration of nitrate and nitrite correlated significantly with volume and sperms activity. These findings are consistent with those of Donnelly *et al.* (1997) who noted that endogenous NO appears to have an important role in the maintenance of sperm motility, if carefully regulated. Similarly, Dambrova *et al.* (2003) noted that NOs

Table 1: Sexual maturity and semen characteristics of Nilotic pubertal bucklings

Variables	SMD	MPD	±SE	LS
Number of animals	5	8		
Puberty weight, kg	10.39	11.16	0.46	NS
Sexual maturity weight, kg	11.2	12.91	0.46	S
Puberty age, Days	179.39	169.75	5.44	NS
Sexual maturity age, days	222.24	225.09	1.63	NS
Reaction time	36.54	30.02	2.36	NS
Volume	0.23	0.39	0.03	S
Mass activity	2.77	3.02	0.13	NS
Individual motility	66.04	70.66	1.43	S
Semen Nitrite mg/100ml	0.04	0.04	0.002	NS
Semen Nitrate mg/100ml	0.04	0.04	0.001	NS
Semen Zinc mg/1ml	0.15	0.15	0.003	NS

SE = Standard error.
LS = Significance level of treatment
NS = Treatment effect is not significant (P>0.05); S = Treatment effect is significant (P<0.05);

Table 2: Development of some semen characteristics in Nilotic pubertal bucklings

Week of ejaculate	Observations No.	Reaction time	Volume	Mass activity	Individual motility	Nitrite	Nitrate	Zinc
1	6	40.0	0.242	2.8^{d}	67.5^{bc}	0.04	0.04	
2	9	34.5	0.234	1.5^{d}	61.3^{c}	0.04	0.05	0.12
3	10	33.0	0.215	2.3^{cd}	61.0^{c}	0.04	0.04	0.14
4	9	32.3	0.247	2.8^{bc}	64.1^{c}	0.06	0.06	0.12
5	11	37.1	0.287	2.6^{c}	62.9^{c}	0.04	0.03	0.13
6	11	41.8	0.358	3.6^{b}	74.7^{b}	0.03	0.04	0.12
7	6	27.5	0.331	2.9^{bc}	68.8^{bc}	0.04	0.04	0.14
Control*	6	20.0	0.431	4.4^{a}	83.6^{a}	0.04	0.04	0.14
SE		4.92	0.056	0.26	2.94	0.003	0.003	0.005
Sign.		NS	NS	S	S	NS	NS	NS

Control* = adult bucks
$^{a, b, c}$ and ^{d} = Means with different superscripts on the same column are significantly (P<0.05) different.

Table 3: Correlation coefficient matrix of some semen characteristics in Nilotic pubertal bucklings

No.	Trait	1	2	3	4	5	6	7
1	Reaction time	1						
2	Volume	-0.07	1					
3	Mass activity	-0.07	0.41*	1				
4	Individual motility	-0.25	0.40*	0.81*	1			
5	Nitrite	-0.21	0.25	0.26*	0.03	1		
6	Nitrate	-0.08	0.27*	0.30*	0.23	0.65*	1	
7	Zinc	0.17	0.24	0.21	0.21	0.04	0.21	1

Traits on rows are those in columns
Number of observations = 59
*= Correlation is significant (P<0.05).

isoforms have been shown to regulate a number of functions including sperm motility and maturation.

This study concluded (i) that Nilotic kids attained puberty by six months (5.8 months) of age, but sexual maturity in seven weeks later at 7.5 months of age with an average body weight of 12 kg, (ii) that the sorghum meshed diet (SMD) can be replaced by molasses pelleted diet (MPD) without affecting the normal growth of pubertal Nilotic bucks and (iii) that the study gave encouraging highlights on the use of seminal biochemical contents as zinc and

nitric oxide levels in semen as indicators for sexual maturity and fertility of bucks. However, this study was based on a rather small number of observations and therefore further study using larger number is needed.

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