

Full Length Research Paper

Semen characteristics of pubertal West Africa dwarf rams fed pineapple waste silage as replacement for dried cassava peel

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In the tropics, ruminants are raised on natural pastures which decline rapidly in quality and quantity during the dry season. This study assessed the reproductive performance of rams fed with cassava-peel-silage (CPS) blended with pineapple-waste (PW) on: scrotal circumference, testicular size and semen volume. Randomized complete block design with four treatments of PW at 0, 20, 40 and 60% w/w in Diets 1, 2, 3 and 4 were used. Each treatment had three replicates while semen was collected once from all replicates in the treatments. Rams were fed experimental diets for 56 days. Cassava-peel was substituted with PW at 0, 20, 40 and 60% w/w in Diets 1, 2, 3 and 4 respectively. Fresh PW were ensiled in plastic mini silos. Samples of freshly processed pineapple waste were taken on the day of ensiling and ensiled products were opened after 21 days to determine their nutritive value. All silage blends had colours from brown to yellow with pH values from 4.0 to 6.8. The scrotal diameter from 16.00cm to 21.40cm and scrotal length from 8.83cm and 12.75cm but did not differ significantly ($P>0.05$). The sperm motility of rams fed diets 4 and 1 was similar to rams fed other diets. Mean sperm volume, percentage livability and percentage sperm morphology were similar across treatments ($P>0.05$). It can be concluded that CPS blended with PW up to 60% showed no adverse effect on semen characteristics and fertility of the rams.

Key words: Semen characteristics, pineapple waste, Brewer's dried grain, corn cob, silage.

INTRODUCTION

In Tropical Africa, ruminants are raised on natural pastures which decline rapidly in quality during the dry season and due to urbanization. Changes in nutritional status result in very irregular growth and marked fluctuations in seasonal weights (Wilson, 1987). Small-scale farmers cannot afford the investments required to establish improved pastures and feed concentrate supplements to alleviate dry season growth checks. Therefore, small-scale farmers rely on browse plants, crop residues and by-products to supplement roadside

grazing during the dry season. These challenges result in reduction of production of certain livestock species like goats, cattle, swine and poultry. The effects of these challenges have reflected on the quality and amount of animal protein available for human consumption in the third world (Smith et al., 2010). The most crucial constraint affecting livestock production in many developing countries is inadequate animal feed resources. Feed shortages, both quantitative and qualitative, limit livestock productivity. Feed cost is not

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only an important concern in the management of national economies, but, a major cost burden of livestock farms, and thus a major strategy to develop the livestock industry in developing countries could be the use of agricultural by-products like pineapple waste, corn cobs and brewers dry grain (Onwuka et al., 1997). One of such agro-industrial by-products is pineapple waste (PW) from pineapple processing. Pineapple waste occurs as pineapple peels and core, making about 40 to 50% of the fresh fruit (Buckle, 1989) and contains mainly sucrose, fructose, glucose and other nutrients (Krueger et al., 1992). FAO (2004) ranked Nigeria among the leading pineapple producing countries with about 800, 000 metric tonnes, therefore, efforts at finding better use for the PW generated from such huge quantities may be important in terms of environmental pollution and waste as potential animal feed resource. Lamidi et al. (2008) found that broiler chickens could tolerate up to 10% PW in their diets without any deleterious effect. Olosunde (2010) reported that WAD sheep could tolerate up to 45% PW but 30% PW was superior when substituted for corn bran. However, it produced adequate results with older pigs weighing over 57 kg at inclusion rates as high as 50%.

At higher levels, weight gain decreases and feed conversion is depressed. Grinding did not improve daily weight gain and feed efficiency therefore, protein supplementation is required (Göhl, 1982). Due to this high variability, pineapple wastes have been described as equivalent to cereal grains for ruminants (Müller, 1978) or as a low-nutrient feed (Hepton and Hodgson, 2003). In any case, the high amount of fibre makes pineapple wastes more suitable to ruminants than to pigs and poultry. The bulkiness of the fresh products limits intake (O'Donovan, 1978). In adult sheep, the inclusion of up to 14% of dehydrated pineapple waste in Napier grass (*Pennisetum purpureum*) silage did not modify nutrient digestibility but linearly increases dry matter (DM) intake (Ferreira et al., 2009). Dried pineapple could be included at up to 27% in the diet (cottonseed meal and maize grain), replacing fresh Napier grass: the highest DM intake were observed at 11% and the authors recommended 16% as an optimal inclusion rate (Rogerio et al., 2007). Babatunde (1988) classified PW as an alternative feed ingredient to conventional wheat offal. These indicate potential for use as animal feed. The objective of this study is to determine the reproductive potentials of West African Dwarf (WAD) rams fed silage blends of pineapple waste, Brewer's dried grain (BDG) and corn cob, thereby investigating the effects of the silages on the spermatozoa attributes and scrotal characteristics of WAD rams.

MATERIALS AND METHODS

Animals and management

In a randomized complete block design, twelve healthy pubertal WAD rams aged six months were divided into four treatments with three replicates per treatment. The rams were obtained as lambs

born to ewes at the Sheep unit of the Teaching and Research Farm of University of Ibadan where the experiment was conducted. The animals were weaned between 63 and 70 days of age with weight ranges between 15.00 and 16.10 kg and housed together in a group in a standard sheep pen with concrete floor till they were six months old. For the period of rearing till six months, they were fed wheat offal based diet concentrate ration supplemented with forage *ad-libitum* and had access to cool clean drinking water at all time. They were allowed out for exercise early in the mornings (0700 h) on days with favourable weather. However, at six months of age, they were subjected to a 56 day growth study to access the effect of pineapple waste silage on their semen after the experimental period. During the trial, animals were given fresh water and salt lick *ad-libitum*. Shown in Table 1 is ingredients (g/100 g) and chemical composition (%) of experimental diets.

Semen collection

Semen was collected once 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-s rest intervals between stimuli, increasing the voltage stimuli by one volt at a time. The penis was prolapsed beyond the prepuce, and semen was collected into a graduated collection vial and analyzed immediately at room temperature. 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. Alternate current increasing in voltage gradually from 0 to 5 volts and returning again to zero within every 5 to 10 s was initially passed. The subsequent stimulation made progressively higher so that at about 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 by electro-ejaculator, the volume of each ejaculate was measured in a graduated tube. The proportion of spermatozoa with an intact apical ridge was evaluated. After fixation in a buffered 2% glutar aldehyde solution and examined under Differential Interference Contrast microscope microscopy at magnification of 400. Total number of spermatozoa per ejaculate was calculated as the product between sperm concentration and volume of the ejaculate. Percentage of abnormal spermatozoa (considering all normal forms in sperm head, intermediate piece and tail) was estimated.

Sperm volume

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 1 ml equals 1 g. Thereby, loss of volume associated with transfer from the collection tube to either another tube or a pipette can be avoided (Jørgensen et al., 1997).

Sperm motility

Sperm motility was assessed by the method described by Zemjanis (1977) and was evaluated microscopically within 2 to 4 min of their isolation from the caudal epididymis and later expressed as

Table 1. Ingredients (g/100 g) and chemical composition (%) of experimental diets.

Ingredient	1	2	3	4
Pineapple waste (PW)	0.00	20.00	40.00	60.00
Dried cassava peel (DCP)	68.00	48.00	28.00	8.00
Brewers dried grain (BDG)	20.00	20.00	20.00	20.00
Ground maize cobs (GMC)	10.00	10.00	10.00	10.00
Urea (U)	2.00	2.00	2.00	2.00
Dry matter	84.57	78.48	68.88	62.59
Crude protein	12.15	12.74	13.42	13.74
Crude fibre	12.44	12.65	13.03	13.68
Ether extract	5.51	4.63	3.75	2.85
Ash	6.06	5.42	4.64	3.58
Nitrogen free extract	63.84	64.56	65.17	66.15
Acid detergent fibre	24.44	26.67	28.58	20.62
Neutral detergent fibre	40.61	43.63	47.20	41.57
Acid detergent lignin	5.46	3.54	3.29	2.45
Hemicellulose	16.17	16.96	18.62	20.95
Cellulose	18.98	23.13	25.29	18.17

percentages. 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).

Morphology

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 (Wells and Awa, 1977).

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

Sperm motility, percentage livability, sperm volume, mass activity and morphology of rams fed silage blends of pineapple waste, Brewer's dried grain (BDG), cassava peels and maize cob presented in Table 2 are the percentage sperm motility, live/dead ratio, sperm volume,

mass activity and morphology of rams fed silage blends of pineapple waste, BDG, cassava peels and maize cob. Scrotal diameter, the mean scrotal diameter in centimeter ranges from 16.00 cm to 21.40 cm. Although, there were variations among the treatments, there was no significant difference ($P > 0.05$) between groups. Scrotal length, the mean scrotal length in centimeter of the rams fed silage blends varies from 8.83cm in Diet 1 and 12.75 cm in Diet 4. The semen volume ranged between 0.01 ml and 0.23 ml. There was no significant difference ($P > 0.05$) between the treatments.

Percentage sperm motility ranged between 66.25 and 85.00%. However, this difference was not statistically significant ($P > 0.05$) between groups throughout the experiment. Percentage livability ranged between 87.50 and 90.00. There was no significant difference ($P > 0.05$) between the treatments throughout the period of the experiment.

The mean mass activity ranged between 3.0 and 3.5. There was no significant between the treatments ($P > 0.05$) in the sperm mass activity throughout the period of the experiment.

DISCUSSION

There was no significant difference ($P < 0.05$) in the mass activity of semen across the different stages of the work. This is similar to the effect of the pumpkin plant on the mass activity of semen of WAD bucks as reported by Oyeyemi et al. (2000). There were no significant differences observed in scrotal length and scrotal circumference. However, this work is inconsistent with those obtained by Fernandez et al. (2004), Hotzel et al. (2003) and Oldham et al. (1978) who found that testicular growth can be affected when animals were fed above

Table 2. Scrotal diameter, scrotal length, sperm motility, live/dead ratio, sperm volume, mass activity, colour and morphology of rams fed silage blends of pineapple waste, BDG, cassava peels and corn cob.

Parameter	Treatments			
	1	2	3	4
Scrotal circumference (cm)	20	19.17	16	21.4
Scrotal length (cm)	8.83	12	9.33	12.75
Sperm motility (%)	85.66	83.25	66.25	85
Livability (%)	87.5	90	84.17	87.66
Sperm volume (mL)	0.12	0.1	0.17	0.23
Mass activity	3.33	3.5	3	3.5
Abnormal Morph. (%)	11.84	14.91	12.47	12.96
Colour	Creamy	Creamy	Creamy	Creamy

^{Abc} means in the same row with different superscripts are different at $P < 0.05$.

their maintenance requirement. The number of dead spermatozoa increased but not significantly ($P > 0.05$) throughout the study. Masters and Fels (1984) reported that testicular size is controlled by nutrition, even to the extent that well-fed rams in spring may have larger testes than poorly-fed rams in autumn. However, the results of the present study depicted that semen volume was not significant ($P > 0.05$) in treatment group. However, the result of this study did not show any trend in semen volume, therefore semen volume was not significant ($P > 0.05$) in treatment group. This is contrary to the report of Kheradmand et al. (2006) that there was a tendency for semen volume to increase ($P = 0.073$) in treatment group, in comparison with the control group. Similarly, this difference was not statistically significant between groups throughout the experiment. Semen volume is one of the important factors in semen evaluation and reproductive performance in the males (Ax et al., 2000).

Although, the differences obtained were not statistically different, semen volume and sperm motility values obtained in this study were respectively lower than the corresponding values reported by Nour et al. (1981) for adult Katjang and Katjang x German Fawn goats. The differences in seminal characteristics could be adduced to breed and species of animals used in these studies. A number of studies have demonstrated that the spermatogenesis in rams is sensitive to increases in protein intake. This effect has been related to an increase in testicular size because it is due to an increase in the volume of seminiferous epithelium and in the diameter of seminiferous tubules, however this result is similar to the report of Hotzel et al. (2003), Abi et al. (1997) and Oldham et al. (1978).

The colour of semen of experimental rams obtained in this study was similar throughout the experiment. This is in accordance to the reports of Bitto et al. (1988) and Oyeyemi et al. (2011), they observed a creamy colour characteristic of WAD buck. In general, total abnormalities per group or as per total sperm cells in all

the groups were within normal range and show that increasing plane of pineapple waste did not have any adverse effect on the sperm count. This conflicts the work of Jibril et al. (2011) who found out that feeding high protein diet (17.94% CP) had a negative effect on semen concentration and resulted in lower motility confirming that feeding high level of CP in diet is associated with decline in fertility. Sperm output, sperm morphology, semen volume and sperm viability were not influenced by level and source of protein.

For all semen characteristics studied, there was no significant difference, which agrees with the work of Zeragoza et al. (2009) who observed no difference between feeding levels and semen characteristics in Payoya bucks. Scrotal circumference is an important indicator when observing animals for breeding soundness. It is favorably correlated to testes mass, sperm production, and semen quality, age at puberty, body weight and age in young bulls (Swanepoel and Heyns, 1990). Animals with small testicles have reduced sperm production and poor semen quality, the animals in this study had decreased proportion of functional seminiferous tubules, reduced sperm output and elevated percentage of morphologically abnormal sperm (Cates, 1975; Coulter and Foote, 1979).

Moreover, nutritional factors, more than any others, readily lend themselves to manipulations to ensure positive outcomes (Smith and Akinbamijo, 2000). As indicated by scrotal diameter from this study, testicular size was not affected by the different silage blends. This result supported the hypothesis obtained by Bielli et al. (1999). Lindsay et al. (1984) found no significant effect ($P > 0.05$) of improved pasture or high dietary protein on testicular dimensions. The volume obtained is similar to that of earlier reports on the WAD bucks by Ajala et al. (2001).

Thompson et al. (1992) reported that scrotal circumference (SC) was not an accurate predictor of sperm morphology or motility when a SC of 32 cm was

used to predict the recommended minimal standards for semen quality. In addition, there was no significant linear relationship between SC and either the degree of germinal epithelial loss or the percentage of Grade 4+ seminiferous tubules in the bulls completing the performance test. Knights et al. (1984) reported a favorable genetic relationship of SC with measures of semen quality and quantity. In general, as SC increases in yearling bulls, mass motility, percentage normal sperm, semen volume, sperm concentration and total sperm output increased while the percentage of sperm abnormalities decreased.

Pineapple waste has no adverse effect on the livability of sperm cells. PW can be included in small ruminant nutrition and in sheep production in particular without any detrimental effect on semen characteristic. PW did not reduce fertility in male animals and can be included in the ration of sheep up to 60% without any detrimental effect on the semen characteristics. Therefore, PW can be utilized in WAD ram production to reduce environmental pollution of the wastes which is always disposed indiscriminately.

In conclusion, pineapple waste and cassava peels (CP) compete favorably with conventional protein sources to increase crude protein of WAD rams. Also, feeding 13.74% CP diet formulated using pineapple waste resulted in higher semen quality especially sperm motility, live/dead and sperm volume.

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