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RUMEN pH AND MICROBIAL POPULATION OF YANKASA RAMS FED ENSILED SUGARCANE WASTE FORTIFIED WITH POULTRY LITTER

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ABSTRACT

Rumen contents may be observed for physical aspects (color, odor, consistency, sedimentation) and the chemical characteristics (pH, glucose, fermentation, nitrate reduction and methylene blue reduction test) and biological parameters (bacteria, protozoa and fungi) (Donato *et al*., 1999). The study was conducted to evaluate the effects of feeding ensiled sugarcane waste (ESCW) fortified with poultry litter (PL) to Yankasa rams on rumen fluid characteristics using 16 rams (mean initial BW 28.94 ± 5.77 kg; aged 12 to 18 months). Sugarcane waste was mixed with poultry litter in 3:1 and ensiled for 21 days, then use compound a complete diet. The rams were allocated to four groups on the basis of body weights into control group without ESCW (CG) as treatment groups (T_1), 15% (T_2), 30% (T_3) and 45% (T_4) in a Completely Randomized Design (CRD) and fed *ad libtum* for 84 days. At the end of the experiment, ten (10) ml of rumen fluid was drawn from individual experimental animal before feeding at 0 hr and 3 hrs and 6 hrs after meal. Parameters observed were pH, rumen bacteria and fungi biomass. The results showed that rumen bacteria and fungi counts were significantly ($P<0.05$) affected by sampling time. Significant ($P<0.05$) differences were observed in the counts between different sampling times. In conclusion, the ESCW had positive effects on the rumen fluid of the rams by improving rumen pH and microbial activity (bacteria and fungi). It is recommended that rumen fluid could be sampled at 3 hours after feeding for higher bacteria and fungi counts.

Keywords: Fungi, Microbes, pH, Sample, Bacteria

INTRODUCTION

Nutrition is a crucial factor militating against livestock production in Nigeria and it is the bed rock of performance in animals. The Natural pasture grazed by ruminants and crop residues from farm lands in northern Nigeria cannot meet the energy and protein requirement of the animals (Ashiru *et al*., 2017). Maigandi *et al*. (2010) reported that the problem of livestock nutrition has further been increased by competition between man and animals for scarce grains, making it very difficult to meet up with the nutritional requirements of animals at affordable cost. In addition to marked loss of weight, low disease resistance and death, there is reduced fertility and retarded growth in young animals due to poor nutrition (Ajayi *et al.*, 2007). Crop residues and agro-industrial by-products used as supplements for ruminant animals is becoming increasingly popular in Nigeria. The suitability of grain offal and crop residues in feeding ruminants is also well documented. Commonly used unconventional feedstuff includes municipal wastes, sugarcane waste, soybean meal residue, kitchen waste, animal excreta, rumen contents, tannery wastes and other products (Boda, l990).

Sugarcane is a tropical grass from which sugar is extracted leaving two by products which are molasses and baggase (fibrous residue). Baggase contains high fiber, low protein and is very in low digestibility (Liu and Guo, 2012). Sugarcane waste is obtained when the cane is being processed for chewing and comprises the top, peels and the baggase (Woolford, 2000). Fresh poultry manure is about 30% crude protein on a dry basis, about half of which derives from uric acid. For ruminants the digestibility of the crude protein is close to 80% and that of the organic matter about 65%. Poultry manure is also rich in minerals, which makes further mineral supplementation of rations containing dried poultry manure unnecessary (Alvarez *et al.*, 2005). Saricicek and Ozel (2010) stated that fluctuation of the number and ratios of rumen microorganisms according to the rumen condition is very important in terms of retaining higher feed efficiency and rising of the amount and quality of feeds. Plant cell walls are degraded by a combination of bacteria, fungi and protozoa, with bacteria and fungi contributing approximately 80% and protozoa 20% of the degradative ability (Euge'ne *et al.*, 2004). This study aimed to evaluate the effects ensiled sugarcane waste fortified with poultry litter on rumen fluid characteristics of Yankasa rams.

MATERIALS AND METHODS

Study Location

The study was carryout at the Teaching and Research Farm of Aliko Dangote University of Science and Technology, Wudil, Kano State.

Collection and preparation of experimental materials

Soybean meal residue (SBMR) was obtained from various soya bean cake (*awara*) processing centers in and around the metropolis. Sugarcane waste (SCW) was collected from processing/selling points identified within and around Wudil local government of Kano State. All materials (stones, iron and polyethene) were removed. Urea feed grade was obtained from market while poultry litter (PL) was obtained from a deep litter poultry production system. The collected SCW, SBMR and PL were sun dried for a period of 3 days during the dry season by thin spreading on a concrete floor. Soybean grain was sourced from the market. The grain was soaked in

water overnight dried by spreading on a concrete floor under sun light for 3 days. After drying it was milled to produce soybean meal (SBM). Other feed ingredients for the experiment which: include maize, wheat offal, cotton seed cake, cowpea husk and salt were obtained from the market.

Rumen fluid collection and pH determination

Ten (10) ml of rumen fluid was drawn individually from all the experimental animals using sanction tube at 0 hour (before feeding) and at 3 hours and 6 hours after feeding. Collected samples were strained through two layers of cheesecloth (Prigge *et al*., 1984), pH value was measured immediately using digital pH meter. The rumen liquor was immediately poured into sterile glass bottles and kept in a flask containing ice blocks and transported to the laboratory for analysis.

Microbial composition of the rumen

The bacteria and fungi biomass in the rumen fluid were estimated at 0, 3 and 6 hours after the morning meal by the method described by Broudiscou *et al*. (1997). The subsamples of rumen fluids obtained were transferred to a tube and centrifuge at 3000 rpm for 5 minutes. Five (5) ml of supernatant from centrifuged rumen fluid in a test tube was taken and 5 ml of 10% formalin solution was added to kill the bacteria. Then 2 ml of formalin mixed rumen liquor in a test tube was transferred and 8 ml of distilled water was added to give 1 x 10^{-1} dilution. Serial dilutions up to 1x 10^{-6} were made on clean glass slide. 0.01 ml of sample from all the dilutions was taken on separate sterile glass slide on a marked area of 2 x 2 cm with of saturated solution of nigrosine on the glass slide. This was then thoroughly mixed and stain with help of loop wire, spread on slide as thin as possible. The slide was kept on hot plate for 2 seconds to dry the smear. Counting was done under oil immersion lens. Bacteria were count per ml of rumen liquor was done by following formula. Ruminal bacteria/ml of rumen liquor = Average number of bacteria per field x microscopic factor (1000) x dilution factor (10^6) (Broudiscou *et al*., 1997).

For fungal counts, one millilitre of the sample added to the fermentation tube to give concentration of 2,000 U of penicillin and 130 U of streptomycin/ml to inhibit bacterial growth. The antibiotics and fungicide were dissolved in distilled water previously gassed with oxygen free carbondioxide, sterilized by passage through a 0.2-µm-pore-diameter polysulfone membrane filter and added anaerobically and aseptically to the individual tubes prior to inoculation. Serial dilutions up to 1x 10^{-6} were made on clean glass slide and calculation done as for bacteria.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS version 9.1 (SAS 2010). Where significant differences were seen, Duncan Multiple Range Test (DMRT) was used to separate the means. The variation between the means was considered at 5% probability level (P<0.05).

RESULTS AND DISCUSSION

Results

The results of rumen fluid characteristics were presented in Table 1. The results showed that rumen bacteria and fungi counts were affected by sampling time. Significant (P<0.05) differences were observed in the counts between different sampling times. The animals had initial bacterial load of 7.38 × 10^6cfu/ml which was later increased to 8.70 × 10^6cfu/ml at three hours post feeding. At 6 hours post feeding, the bacterial count was reduced to 6.74 × 10^6cfu/ml. Results for fungal counts showed that at 0-hour (before feeding) sampling time, the value obtained was significantly (P<0.05) higher than the values recorded at 3 and 6 hours post feeding.

Table 1: pH, bacteria and fungi count of Yankasa rams fed graded levels of ensiled sugarcane waste (ESCW) fortified with poultry litter (PL)

Parameters	Sampling Time (hrs)	Treatments				LSD
		T_I (0%)	T_{II} (15%)	T_{III} (30%)	T_{IV} (45%)	
Ph	0	7.46	7.61	7.71	7.67	0.908
	3	5.53	5.58	5.63	5.90	0.783
	6	6.95	6.55	6.52	6.94	0.886
Bacteria ×10^6cpu/ml	0	7.30	7.28	7.60	7.32	1.001
	3	8.17^b	8.80^{ab}	8.97^a	8.87^{ab}	0.724
	6	7.24^a	6.73^{ab}	6.35^{bc}	5.98^c	0.654
Fungi ×10^6cpu/ml	0	6.88^b	6.67^b	8.85^a	6.68^b	1.106
	3	7.38^a	6.31^b	6.20^b	7.25^a	0.850
	6	6.71	6.68	6.73	6.83	1.128

[a,b,c]Means with different superscripts within the same row are significantly different (P<0.05). cpu/ml = colony forming unit per mililiter, LSD = Least Significant differences

Table 2: Bacteria and fungi counts of Yankasa rams fed graded of levels ensiled sugarcane waste (ESCW) fortified with poultry litter (PL) based on sampling time

Parameters	Sampling Time (hours)			LSD
	0	3	6	
Bacteria ×10^6cpu/ml	7.38^b	8.70^a	6.74^c	0.432
Fungi ×10^6cpu/ml	7.27^a	6.78^b	6.57^b	0.398

[a,b,c]Means with different superscripts within the same row are significantly different (P<0.05). cpu/ml = colony forming unit per mililiter, LSD = Least Significant differences

Discussion

Rumen pH is one of the most important factors affecting fermentation in the rumen and influences its function. It is of interest to note that all the treatments showed non-significant neutral pH values before feeding (zero hours). This result may suggest that the poultry litter used to treat sugarcane waste contained available N and may provide more nutrient which serve as appropriate media for ruminal microflora and in turn led to enhanced activity of the microbes. The pH values obtained before feeding in the present study were similar to values (7.08 – 7.34) for lactating Red Sokoto goats fed dietary levels of palm oil (Otaru *et al*., 2010). Maekawa *et al*. (2002a) stated that rumen pH generally declines after feeding. The pH values of all the treatments decreases three hours after feeding, the decrease in pH was dependent on the initial pH (Maekawa *et al*., 2002a) the lowest pH generally occurring four to six (4 – 6) hours after feeding (Nocek *et al*., 2002). Otaru *et al*. (2010) reported a decrease in pH levels (6.53 – 6.85) after feeding.

Plant cell walls are degraded by a combination of bacteria, fungi and protozoa, with bacteria and fungi contributing approximately 80% of the degradative activity, and protozoa 20% (Dijkstra and Tamminga (1995). The increase in bacterial count at 3 hours after feeding might be due to bacterial activities in the rumen which resulted in the conversion of feeds to VFAs (Bonhomme, 1990). The association and attachment of rumen microbes to feed particles by ruminal microorganisms is rapid occurring within five minutes of feed entering the rumen (Bonhomme, 1990). The decrease in bacterial count at 6 hours sampling time might be due to the fact that microorganisms themselves are digested to free up nutrient for the ruminants use (Ensminger, 2002). Dehority and Tirabasso (2001) observed that bacterial count was not affected by sampling period.

Fungi are the first organisms to invade and commence digesting the structural plant components, from the inside, they reduce the tensile strength of these particles and thus increase particle breakdown in rumination (Akin *et al*., 1989). The fungi count was observed to be statistically different at 0 hours sampling time. The process might be attributed to the fungi's efficient enzymes system which can degrade structural elements of the plant cell wall. The results of this study indicated that there were no deficiencies in nitrogen, the pH of the rumen fluid of the animals were optimum for fibre digestion. The results also indicated that the number of bacteria and fungi in solution were adequate for fibre degradation. This is in agreement with the findings of Ørskov (1994) who observed that not only deficient nutrients like nitrogen and sulphur and low pH can limit degradation rate but also that the number of microbes in solution can be a limiting factor particularly for very poor-quality roughages. Ørskov (1994) further concludes that optimal feed utilization and health of the animals can be achieved by ensuring optimal condition for cellulolysis in the rumen.

CONCLUSION

In could be concluded that ESCW had positive effects on the rumen fluid of the rams by improving rumen pH and microbial (bacteria and fungi) activity.

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