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A Study on Rumen Cilliate Protozoa Population, pH And Some Metabolities in West African Dwarf (WAD) Sheep Fed Forage and Concentrate Diets

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[1]**Target Audience:** Ruminant nutritionist, reuminologists, sheep and goat scientists and farmers.

Abstract

An investigation on rumen ciliate protozoa population, pH and some metabolites (total volatile fatty acids, rumen ammonia Nitrogen) was conducted on two fistulated WAD rams fed forage and concentrate diets. The 12-week study focused on the sequence of production of these parameters under each dietary regime.

The diets fed were a grass sword consisting mainly of Panicum maximum and containing 6.50% crude protein (CP) and a concentrate ration formulated from maize, groundnut cake, fish dust meal, brewers dried grain, bone ash and common salt and containing 12.08% crude protein (CP). The diet were fed consecutively for 6 weeks and rumen sampling was carried out in the last 4 weeks of each feeding regimen. Rumen liquor samples were drawn hourly in batches during morning (9am-12noon) and afternoon (1pm-4pm) periods on each sampling day. Rumen pH was recorded for each hourly collected sample before pooling by batch. Each pooled batch of samples was estimated for ciliate protozoa population(CPP) and subsequently analysed for total volatile fatty acids (TVFA) and rumen ammonia nitrogen (RAN).

The results showed that nutritional regime significantly ($P<0.05$) influenced rumen pH and concentrations of TVFA, RAN and CPP. These parameters were also affected ($P<0.05$) by periods of sampling. Significant positive correlation existed between rumen pH and RAN ($P<0.01$, $r=0.74$); rumen pH and CPP ($P<0.01$,$r=0.87$) and RAN and CPP ($P<0.01$, $r=0.77$), while the relationship between rumen pH and TVFA ($P<0.01$, $r=0.84$); RAN and TVFA ($P<0.05$, $r=-0.39$ and CPP and TVFA ($P<0.01$, $r=-0.84$); were negative and significant. These results confirm that TVFA, RAN and CPP are true parameters for predicting the nutritional status of ruminant animals at any given time.

Keywords: Rumen pH, cilitate protozoa population, rumen ammonia nitrogen, total volatile fatty acids fistulation, dietary regime.

Description of Problem

Volatile fatty acids (VFA), rumen ammonia nitrogen (RAN), ciliate protozoa population (CPP) and rumen pH are all components of the ruminal fluid. They also constitute the indices for evaluating the nutritional status of the ruminant animal(1)

VFAs are produced from dietary carbonhydrate metabolism in the rumen (1)and also by the action of rumen microbes as they degrade the complex polysaccharides(cellulose, hemi-cellulose) of fibrous feed components. These VFAs (acetic, propionic and butyric) so produced supply 55-60% of the energy requirements of ruminants (2), even though the proportions have little effect on the efficiency of the energy utilization (3). Rumen (15). However, there is probably no single test that can disclose the nutritional status of the ruminant as does protozoa population in the rumen (15).

Rumen pH measures the acidity of the ruminal fluid and is greatly influenced by nutritional regime and consequently the concentration of VFAs in the rumen. This study was designed to evaluate the influence of dietary regime on rumen pH, CPP, as well as the production of TVFA and RAN in sheep monitored within specific (morning and afternoon) sampling periods.

Materials and Methods

Animal Management:

Two fistulated WAD rams aged between 15-16 months and averaging 18.20kg in body weight were housed individually in well ventilated cement floored pens at the University of Nigeria Veterinary Teaching Hospital, Nsukka. Each pen was provided with a feeder and waterer. Prior to fistulation, the animals were given acaricide bath and dewormed with Fenbendazole. Fresh forage and concentrate diets (Table 1) were offered consecutively for 6 weeks. Weighted quantities of each diet in excess of 3% body weight dry matter consumption (1kg concentrate or 2kg forage) were offered at 0800-0900 hrs each day. Daily feed intake and weekly body weights were recorded for each animal. Drinking water was also provided liberally, but were usually removed during sampling hours, on each sampling day, so as not to interfere with the production and estimation of this parameters with time after feeding.

Rumen sampling:

Rumen liquor (sample) collection took place weekly in the last 4 weeks of each feeding regimen. Samples were drawn with the aid of a stomach tube using sunction. A total of 4 collections were made per animal and per diet. On each sampling day, rumen liquor was drawn in two batches one in the morning and the other in the afternoon. Each batch was made up of 4 samples drawn hourly either for morning (9am - 12 noon) or afternoon (1pm-4pm) period respectively. Each batch of samples was eventually pooled.

Determinations:

Prior to pooling, rumen pH was recorded for each hourly collected sample and averages within periods noted. Each pooled batch was then estimated for CPP, analyzed for TVFA and RAN concentration as in (16).

Table 1. Ingredients and nutrients composition of the concentrate and forage diets.

Ingredients	Percentage	
	Concentrate	Forage sword*
Maize	80.00	
Groundnut cake	5.00	
Fish meal dust	3.00	
Brewers dried grain	10.50	
Bone ash	1.00	
Salt	0.50	
Nutrient composition		
Dry matter	95.90	45.72
Crude protein	12.08	6.50
Crude fibre	5.19	32.70
Ether extract	5.75	6.91
N-free extract	71.04	46.10
Ash	5.94	7.79

Mainly *Panicum maximum*

Feed Analysis:

The forage and concentrate diets were analyzed for proximate components using (17) methods.

Statistical Analysis:

The means and standard errors of the study parameters were calculated. The study was designed as a 2 x 2 factorial experiment. Factor A, the diet, had two levels-the forage and concentrate diets, while B, the sampling period, also had two levels-Morning and afternoon (given a total of 4 treatment combinations). Each set of parameter observations on study animals per diet and per period, were considered as treatment levels, while the weekly determinations (2x4=8 in all per parameter) were treated as replicates. The parameter values for both animals were pooled since both were of same breath and fairly of same age and weight. The data generated was subjected to analysis of variance (18) while significant means were separated using (19). Also simple correlation and regression analysis was used to determine the nature and degree of relationships among study parameters.

Results and Discussion

The mean values for rumen pH, CPP, RAN and TVFA in sheep fed forage and concentrate diets and monitored within two sampling periods (morning and afternoon) are summarized in Table 2.

Rumen pH generally declined with time after feeding for both the forage and concentrate diets. Similar observation was reported by (20). They attributed this to increase in the concentrations of TVFA which lowers pH with time after feeding. The pH range of 6.0 - 7.1 obtained for the forage samples in this study was normal for what has been reported (21, 22, 7) for roughages. The same reports however differed from what was obtained for the concentrate samples (5.6-6.3). The reports observed that rumen fluid of all concentrate fed young ruminants remained below 6.0 and above 6.0 for roughage diet. It is possible that the short duration of feeding as well as the limited quantity of concentrate fed may account for the non-conformity. These authors above fed concentrate diets, variously, in excess of 8 weeks

Table 2. Rumen pH, ciliate protozoa population and rumen metabolites in sheep fed forage and concentrate diets.

Period	Parameters	Forage	Concentrate
Morning	rumen pH	6.75 ± 0.03^a	6.07 ± 0.02^b
	RAN(m/ML	5.46 ± 0.05^b	7.50 ± 0.09^a
	TVFA(Meq/100ml)	6.14 ± 0.16^b	6.53 ± 0.05^a
	CPP($X10^5$/ml)	3.09 ± 0.08^a	0.97 ± 0.01^b
Afternoon	rumen pH	6.21 ± 0.02^a	5.74 ± 0.02^b
	RAN(mM/L)	4.55 ± 0.08^b	6.28 ± 0.06^a
	TVFA(Meq/100)	7.44 ± 0.08^b	8.14 ± 0.04^a
	CPP($X10^5$/ml)	2.54 ± 0.07^a	0.87 ± 0.01^b

[ab] Means on the same row with different superscripts differ significantly($P<0.05$).

Within morning and afternoon periods (Table 2), average rumen pH values signififcantly ($P<0.05$) differed among diets. The values were higher for the forage than concentrate samples. Concentrate diets have been known to readily promote the production of VFA, which invariably lowers the rumen pH(23). Between periods, rumen pH was significantly higher ($P<0.05$) for each diet, for samples drawn in the morning than afternoon confirming that rumen pH actually decreases with time after feeding (20).(3) corroborated this view and further

explained that TVFA production reaches its peak at about 6hours after feeding, and remained considerably constant thereafter.

Rumen ammonia nitrogen concentration also differed (P<0.05) between diets within periods. The value were higher for the concentrate than forage samples drawn both in the morning and afternoon. The significant ammonia-N-concentration recorded with the concentrate diets this study may related to its higher CP content. Reports(2,5) have shown that feeding concentrate diets results in more easily and ready production of rumen ammonia (nitrogen) than forages, due to their relatively higher CP content. Between periods, RAN concentration was superior (P<0.05) for each diet, for samples drawn in the afternoon than morning. (24) reported similar findings. According to (25) this could be due to the fact that the rate of deamination of feed protein was slower than proteolysis and that immediately after feeding, there was increased concentration of amino acids and peptides in the rumen, and that subsequent deamination increased the rumen ammonia nitrogen concentration which is at peak 4-5 hours post feeding. Meanwhile the range of values for RAN obtained for the forage (4.3-5.6 millimoles/litre of digesta) and concentrate (6.2-7.9 millimoles/liter) based samples fall within the optimal range reported for microbial activity (26,27,24,4).

TVFA production was higher (P<0.05) for the concentrate than the forage sample collected within both morning and afternoon period. This observation is in line with the findings of (28) that concentrate diets readily promote the production of VFA than forage diets and this has been attributed to their more digestible nature (29). The concentrate diet in this study was more digestible than the forage diet (83.3% vs 69.13%). More TVFA was produced (P<0.05) during the afternoon than morning period and this was true for both diets. The time of 6 hours reported by (3) as the peak production of TVFA in ruminants post feeding, however agrees with the afternoon period of collection. The range of TVFA production of 5.5-7.8 and 6.3-8.3 mill-equivalent per 100ml of digesta(Meq/100ml) recorded respectively for the forage and concentrate samples are however in consonance with results of earlier investigations (21,22,16).

The ciliate protozoa population for the forage sample (2.54-3.09 x 10^5/ml of digesta) was normal while (0.01-0.08 x 10^5/ml of digesta) was low for

Table 3. Simple regression equation and correlation coefficients between rumen pH, ciliate protozoa population and rumen metabolites.

Parameters	Prediction equation	SE	R2	R	Sign.
Level					
PH and RAN	Y = -7.63+2.19x 0.28	0.54	0.74	**	
PH and TVFA	Y = 18.82-1.89x	0.13	0.66 -	0.81	**
PH and CPP	Y = -12.45 + 2.31x	0.11	0.77	0.87	**
RAN and TVFA	Y = 8.90-0.303x	0.11	0.15 -	0.39	*
RAN and CPP	Y = 5.96-0.69x	0.07	0.60	0.77	**
TVFA and CPP	Y = 6.20-0.61 x	0.16	0.03 -	0.55	**

* = P<0.05, **=P<0.01

the concentrate sample (23). It is possible that the ciliate protozoa population found in these animals were preponderant of the *Entodinia* and *Diplodinium* species which are affected by diets which promote even transiently low pH (10). In both the morning and afternoon periods, CPP

was significantly higher ($P<0.05$) for forage than concentrate based samples. The reason for this had been earlier adduced(23) Also, the superior ($P<0.0$-5) CPP recorded during the morning period for both diets may not be unconnected with the increased production of TVFA with time after feeding which will trend-wise influence the proliferation of CPP in the rumen.

There were interactions between diets and periods in the rumen pH values recorded ($p<0.01$), RAN produced ($p<0.05$), as well as the CPP observed ($p<0.01$) in this study. This would indicate that there were differences in the rumen pH, RAN and CPP values recorded between diets when compared withibnperiods. Certainly in this study, rumen pH, values were significantly higher for the forage than the concentrate diet in the morning than in the afternoon period. For RAN, the values were significantly higher for the concentrate than forage diet in the morning than in the afternoon period and for CPG the values were significantly better and for the forage than concentrate diet in the morning than in the afternoon period (Table 2).

Regression Analysis

The relationships between rumen pH, CPP, RAN concentration and TVFA are summarized in Table 3. Significant positive correlations existed between rumen pH and RAN ($P<0.01$, $r = 0.74$), rumen pH and CPP ($P <0.01$, or = 0.87) and RAN and CPP ($P<0.01$, $r = 0.77$). TVFA had a significant negative correlation with rumen pH ($P<0.01$, $r = -0.81$), RAN ($P<0.05$, $r =-0.39$) and CPP ($P<0.01$, $r =-0.55$). Previous investigations have established the observed linear relationships between rumen pH and RAN (30), rumen pH and CPP (23) and RAN and CPP (24) as well as the inverse relationships between TVFA and rumen pH (31,22), TVFA and RAN (20) and TVFA and CPP (4) as in this study.

The linear relationship between rumen pH and RAN demonstrates that the concentration of ammonia nitrogen in the rumen at any given time was dependent on rumen pH. At low pH, ammonia - N absorption from the rumen was usually reduced because of its existence in ionized form. At high pH, however, rumen ammonia - N concentration increased but a preponderant amount existed in gaseous form - a fact which makes it easier to be lost through rumen wall (30). For rumen pH and CPP, the linear relationship butteresses the sensitivity of CPP to rumen pH. At high rumen pH, ciliate protozoa population proliferates, but declines as the pH declines (22). The positive relationship between CPP and RAN corroborates the evidence that CPP thrives well in ammonia nitrogen rich rumen environment and vice versa (24).

The inverse relationships, between TVFA and CPP, TVFA and rumen pH highlights the dependence of pH and CPP on TVFA. As the latter increases, rumen pH and CPP decrease. The converse is also true. The negative relationship between TVFA and RAN is explained by the fact that, though both metabolites increased with time after feeding, both are respectively absorbed at high and low rumen pH. Therefore depending on the prevailing rumen pH, the concentration of one will significantly be higher than the other (20).

Conclusion and Recommendation

In conclusion, rumen pH, ciliate protozoa population, total volatile fatty acids and rumen ammonia nitrogen are indeed true indices for prediction or estimating the nutritional status of the ruminant animal. Also the levels or concentrations of these parameters found in the rumen, at any given time, would to a large extent, depend on the type of nutrition the animal is exposed to, as well as, on the length of time after exposure (feeding).

Owing to constraint posed by dearth or basic analytical tools and equipment, individual VFA's and ciliate protozoa species could not be isolated in this study. It is, however, recommended that further studies should be carried out to separate these parameters (TVFA and CPP) and monitor them hourly (and not periodically as was the case in this study) along side rumen pH and rumen ammonia nitrogen. This would indeed give a more definitive picture on the sequence of production of these parameters with time after feeding.

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