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Rumen Metabolites and Microbiome of Semi-intensively Managed West African Dwarf Goats Supplemented Concentrate Diet of Varying Levels of Sodium Humate

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POOR quality forages such as mature grass could be improved by physical, chemical and biological treatment. One of such procedure is supplementing with feed that will modify the rumen environment to improve forage utilization. A 90-day study was conducted to examine the rumen ecology of semi-intensively raised West African Dwarf (WAD) goats fed supplemented concentrate diet containing incremental levels of sodium humate. The thirty (30) WAD bucks (age 10-15 months) used in this study were randomly assigned to five dietary treatments containing 0, 5, 7.5, 10 and 12.5 g/kg diet of sodium humate in a completely randomized design. Data were obtained on rumen pH, ammonia nitrogen, total volatile fatty acids (VFA) and some of their various proportions (Acetate, propionate and butyrate), protozoa, fungi and bacteria counts and were statistically analysed using the GLM procedure of SPSS (version 23). Results revealed that pH, NH3-N, and fungi count were affected ($p<0.05$) by the inclusion of sodium humate in the diets. However, volatile fatty acids and the various proportions, protozoa and bacteria counts were not affected ($p>0.05$) by the inclusion of sodium humate in the diets. It was concluded that sodium humate could be used in the diet of semi-intensively managed WAD goats to stabilize the rumen pH and that levels up to 10 g/kg diet could be utilized to improve fungi count.

Keywords: WAD goats, Sodium humate, pH, VFA, Microbial count.

Introduction

The semi-intensive system has been reported to be the most common system of production for West African Dwarf (WAD) goats in most part of sub-Saharan Africa [1], with improved performance when compared to the extensive system and less labour input in comparison to intensive system. Given the above scenario, it cannot be said that this system is without challenges. One of such challenges is the poor quality of the forages these animals graze given that they become lignified as the season progresses from the rainy to the dry seasons.. This has posed to be a challenge on the performance of the animals. Devendra [2] has reported that the cause of low production rate from local goats is primarily due to poor feeding practices and lack of intensification of the production system have reported it. Feeding practices aimed at improving the utilization of these forages will help to improve the performance of the animals.

Poor quality forages such as mature grass, may be improved by physical, chemical or biological treatment. One of such procedure is supplementing with feed materials that will modify the rumen environment in order to improve utilization of the forages. Extensive research has been performed on the effectiveness of feed supplements that

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will modify the rumen environment in order to improve nutrient utilization and results have been reported for numbers of feed supplements including plant bioactive compounds, dietary lipids, exogenous enzymes, and direct-fed microbials to manipulate ruminal fermentation and improve nutrient utilization in ruminants [3-9]. Few researches on the use of humic acid to modify rumen environment have also been reported [10-14].

Humic substances have antimicrobial properties [15] and such properties have been reported to promote microbial growth [16]. These actions of humic acid in the soil are proposed to have similar responses within the rumen, which could potentially enhance fermentation and digestibility of nutrients through increased microbial activity [17]. Lower ruminal cellulytic activity has been reported to decrease volatile fatty acids (VFA) at addition of humic acids [18]. On the other hand, VFA concentrations (mM), or molar proportions of acetate, propionate, butyrate or valerate, were not influenced by the use of incremental levels of humates in the diet of steers [12]. Increased protozoa count and reduced ammonia concentration was observed with use of humic substances [14] but such actions on protozoa may be inhibited if high concentrations of humates are used [19].

It should be noted that these researches on the modifier effect of humic acids on the rumen environment has been used either *in vitro* or intensive feeding practices [14, 20] which selects forages that are given to the animals. Focus should be to animals that are managed semi-intensively which usually forage on their own. This research therefore seeks to assess the rumen metabolites and microbial counts of semi-intensively managed WAD goats supplemented increasing levels of sodium humate.

Materials and Methods

Experimental site

The feeding trial was carried out at the Small Ruminant Experimental Unit of Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Nigeria.

Ingredient collection and formulation of diet

Sodium humates was obtained from a reputable Company in China. Other feedstuff used in formulating experimental diets were sourced Maize, wheat offal, palm kernel cake, bone meal, mineral premix and salt were purchased from a feed shop in Abeokuta, Ogun state, Nigeria. The ingredients were milled into coarse form and mixed together to form concentrate diet to contain 0, 5, 7.5, 10 and 12.5 g/kg diet of sodium humates, respectively (Table 1).

TABLE 1. Gross Composition (%) of experimental concentrate diets

Parameter (kg)	[2] Treatments				
	Control	5HNa	7.5HNa	10HNa	12.5HNa
Maize offal	30	30	30	30	30
Wheat offal	34	34	34	34	34
Palm kernel cake	32	32	32	32	32
Bone meal	3	3	3	3	3
Vitamin premix	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
**HNa	-	0.5	0.75	1	1.25
Total	100	100	100	100	100
[1] Determined analysis					
Dry matter	88.00	88.00	88.00	88.00	89.00
Crude protein	14.88	14.01	14.35	14.13	14.61
Crude fibre	9.50	9.50	10.00	9.00	10.00
Ash	5.00	5.40	5.45	6.00	6.50
Ether extract	6.50	8.00	7.50	8.00	7.56
NDF	64.00	65.00	63.00	54.00	55.00
ADF	22.00	23.00	19.00	23.00	20.00
ADL	9.00	8.50	8.00	9.00	7.00

[1]NDF-neutral detergent fibre, ADF-acid detergent fibre, ADL-acid detergent lignin

[2]5HNa-5 g/kg diet sodium humate inclusion, 7.5HNa-7.5 g/kg diet sodium humate inclusion, 10HNa-10 g/kg diet sodium humate inclusion, 12.5HNa-12.5 g/kg diet sodium humate inclusion

Experimental animal management and diet

A total of thirty (30) West African Dwarf bucks aged between 10 - 15 months, with weight ranging between 6-8 kg were purchased from local farmers from Abeokuta and environs. Oxytetracycline LA (1 ml/10 kg) was administered to the animals for prophylactic treatment against bacterial disease while Ivermectin LA (1 ml/50 kg) was administered against internal and external parasite infestation. The animals were allotted to a 1 m^2 pen, meanwhile, they were allowed to graze together 6 hrs daily (9:00 am – 3.00 pm) within a confined area containing sown *Panicum maximun*. On return, they were supplemented concentrate (experimental diet) at 4% of their body weight. Water was provided *ad-libitum.* All experimental and animal management procedures were as approved by Ethics Committee of College of Animal Science and Livestock production, Federal University of Agriculture, Abeokuta, Nigeria (ethical clearance number COLANIM/APH/PG/14/0107).

The treatment diets were as follow:

- 0 g/kg diet of sodium humate supplementation
- 5 g/kg diet of sodium humate supplementation
- 7.5 g/kg diet of sodium humate supplementation
- 10 g/kg diet of sodium humate supplementation
- 12.5g/kg diet of sodium humate supplementation

Data Collection

On the day of rumen collection, the animals were not allowed to graze but were fed both panicum maximum and experimental concentrate diets in the individual pens. Rumen samples were collected 6 hours post feeding from the animals at 30, 60 and 90 days of supplementation using a suction tube. The samples were immediately measured for pH using a portable pH meter (Universal PH Test Kit – Digital PH Meter®) and were thereafter filtered with four-layer cheesecloth and subsamples were divided into 3 portions. The first portion will be transferred into plastic bottle to which 5 ml of 1M H_2SO_4 had been added to stop microbial fermentation and then centrifuged at 3,000 xg for 10 min. About 25 ml of the supernatant will then collected and analysed for NH_3-N according to AOAC [21] method. The second subsample was used to determine total volatile fatty acid (VFA) and the proportions of acetate, propionate and butyrate as illustrated by Samuel et al [22]. Third portion was used for microbial count. Protozoa count was obtained by direct observation using a microscope at 10× magnification [23]. Colony forming units/ml (CFU/ml) of both bacterial and fungi were obtained with the pour plate technique using nutrient algae and potato dextrose agar, respectively. The plates were incubated for 24 hours at 37°C. All colonies appearing at the end of the incubation period were counted using digital illuminated colony counter.

Statistical analysis

The pH, ammonia nitrogen, total volatile fatty acids and the various proportions, protozoa, bacterial and fungi counts were analysed using one-way analysis of variance as contained in the general linear models procedures of SPSS (version 23). Significant differences among treatment means where applicable were seperated using the GLM procedure of SPSS. Probability significance was declared at $P \leq 0.05$.

Results

Rumen metabolites of semi-intensively raised WAD goats supplemented with concentrate diet containing incremental levels of sodium humate

Rumen fermentation metabolites of semi-intensively managed WAD goats supplemented with concentrate diet containing incremental levels of sodium humate is shown in Table 2. Higher levels of sodium humates were said to increase the pH of the rumen at 60 days of supplementation, but the pH values were not influenced ($p<0.05$) by inclusion of incremental levels of sodium humate at 30 and 90 days of supplementation. Higher levels of sodium humate were observed to reduce ammonia nitrogen concentration in the rumen at 30 and 60 days of supplementation, however, this effect was not seen at 90 days of supplementation. Rumen ammonia nitrogen was higher ($p<0.05$) in the control, 5HNa and 7.5HNa groups (24.95, 26.09 and 22.68 g/100 ml, respectively) but reduced in the 10HNa and 12.5HNa groups during 30 days of feeding. At 60 days of supplementing incremental levels of sodium humate, ammonia nitrogen was higher ($p<0.05$) in control, 5HNa and 10HNa groups (12.48, 16.44 and 11.34 g/100 mol, respectively), with a reduction ($p<0.05$) in the 7.5HNa and 12.5HNa groups. Total volatile fatty acids (VFAs), acetate, propionate, butyrate and acetate: propionate ratio were not ($p>0.05$) affected by the inclusion of incremental levels of sodium humate in the diet in all the duration of supplementation.

TABLE 2a. Rumen metabolites of semi-intensively raised WAD goats supplemented with concentrate diet containing incremental levels of sodium humate

[1]Parameter	Duration	[2] Treatment groups						[3]Polynomial contrast		
		Control	5HNa	7.5HNa	10HNa	12.5HNa	SEM	L	Q	C
pH	30 days	6.30	6.23	6.63	6.26	6.50	0.07	NS	NS	NS
	60 days	6.20^{b}	6.17^{b}	6.70^{ab}	6.63^{ab}	6.80^{a}	0.10	*	NS	NS
	90 days	6.57	6.63	6.97	6.83	6.43	0.09	NS	NS	NS
NH_3-N (g/100 ml)	30 days	24.95^{ab}	26.09^{a}	22.68^{abc}	19.28^{c}	21.54^{bc}	0.81	**	NS	*
	60 days	12.48^{ab}	16.44^{a}	8.50^{b}	11.34^{ab}	10.21^{b}	0.87	*	NS	NS
	90 days	17.51	23.25	15.88	17.58	18.14	1.20	NS	NS	NS
Volatile fatty acids (Mm/100 mol)	30 days	5.19	5.47	5.19	4.67	5.48	0.13	NS	NS	NS
	60 days	7.48	6.33	6.67	6.46	7.34	0.17	NS	NS	NS
	90 days	5.04	4.74	5.11	5.00	5.08	0.09	NS	NS	NS

[a,b,e]Means with different superscript along the raw differ significantly ($p<0.05$);
[1]NH_3-N- ammonia nitrogen; [2] 5HNa-5 g/kg diet sodium humate inclusion, 7.5HNa-7.5 g/kg diet sodium humate inclusion, 10HNa-10 g/kg diet sodium humate inclusion, 12.5HNa-12.5 g/kg diet sodium humate inclusion; [3]L-linear, Q-quadratic, C-cubic

TABLE 2b: Rumen metabolites of semi-intensively raised WAD goats supplemented with concentrate diet containing incremental levels of sodium humate

[1]Parameter	Duration	[2] Treatment groups						Polynomial contrast		
		Control	5HNa	7.5HNa	10HNa	12.5HNa	SEM	L	Q	C
Acetate (mol/100mol)	30 days	3.46	3.72	3.45	3.11	3.65	0.09	NS	NS	NS
	60 days	4.99	4.22	4.45	4.31	4.89	0.11	NS	NS	NS
	90 days	3.36	3.16	3.07	3.33	3.39	0.07	NS	NS	NS
Proprionate (mol/100mol)	30 days	2.31	2.43	2.31	2.07	2.43	0.06	NS	NS	NS
	60 days	3.33	2.81	3.01	2.87	3.44	0.09	NS	NS	NS
	90 days	2.24	2.11	2.27	2.23	2.25	0.04	NS	NS	NS
Butyrate (mol/100mol)	30 days	0.35	0.37	0.35	0.31	0.37	0.01	NS	NS	NS
	60 days	0.50	0.42	0.45	0.43	0.49	0.01	NS	NS	NS
	90 days	0.34	0.32	0.34	0.33	0.34	0.006	NS	NS	NS
A:P	30 days	1.50	1.53	1.50	1.50	1.50	0.01	NS	NS	NS
	60 days	1.50	1.50	1.48	1.50	1.43	0.01	NS	NS	NS
	90 days	1.50	1.50	1.36	1.49	1.50	0.03	NS	NS	NS

[1]A:P-acetate: propionate ratio; [2] 5HNa-5 g/kg diet sodium humate inclusion, 7.5HNa-7.5 g/kg diet sodium humate inclusion, 10HNa-10 g/kg diet sodium humate inclusion, 12.5HNa-12.5 g/kg diet sodium humate inclusion; [3]L-linear, Q-quadratic, C-cubic

Rumen microbial counts of semi-intensively managed WAD goats supplemented concentrate diet containing incremental levels of sodium humate.

Table 3 shows the rumen microbial counts of semi-intensively managed WAD goats supplemented concentrate diet containing incremental levels of sodium humate. During the first 30 days of supplementation, fungi count was significantly ($p<0.05$) increased in 5HNa, 7.5HNa and 10HNa groups (17.47, 19.05 and 48.93 X 10^3 cfu/ml, respectively) and this reduced in the control and 12.5HNa groups (6.73 and 7.36 X10^3 cfu/ml, respectively). However, at 60 and 90 days of supplementation, fungi count was not affected ($p>0.05$) by the levels of sodium humate supplementation. Protozoa and bacterial counts were not affected ($p>0.05$) by the levels of supplementing sodium humate throughout the period of the experiment.

Discussion

Rumen pH is very vital for tenacity and stability of the gut microbiota. The pH level of the rumen is important because fibrolytic bacteria are very sensitive towards pH changes [24]. Tolerable level of pH for fibrolytic bacterial is between 6.0 and 7.0 [25]. Van Soest [26] has reported optimal ruminal pH ranges to be 6.10-6.80 for rumen microbial proliferation. The higher ruminal pH values of the goats on sodium humate could be a reflection of its buffering capacity. This will possibly ensure stabilization of ruminal acidity, thereby ensuring changes towards improving efficiency of ruminal microbial functions. In addition, rate of absorption of short chain fatty acids could contributes to the stabilization of ruminal pH [27]. This result of pH in this study is consistent with that of El-Zaiat et al. [28] for Baki goats fed humic acids. On a contrary, humic/fulvic acid did not affect pH in finishing steers, rams and heifers [10, 11, 14]. The differences between this current study and that of others could be adjured to specie difference and management practices adopted. The linear decrease in concentration of ammonia nitrogen in the rumen is consistent with reports of El-Zaiat et al. [28] in goats, McMurphy et al. [10] in steers. The strong nitrogen-binding activities of humic acids are reported to be responsible for the reduction in ammonia [10]. In addition, dietary humic acid may enhance crude protein utilization through reduced solubility under the inhibitory action of humic acids on

TABLE 3. Rumen microbial counts of semi-intensively managed WAD goats supplemented concentrate diet containing incremental levels of sodium humate

Parameter	Duration	[1]Treatments					SEM	[2]Polynomialontrast		
		Control	5HNa	7.5HNa	10HNa	12.5HNa		L	Q	C
Protozoa (X10^3 cell/ml)	30 days	46.33	43.67	46.33	50.67	34.00	3.10	NS	NS	NS
	60 days	20.57	17.17	7.70	6.53	1.53	3.70	NS	NS	NS
	90 days	43.67	37.67	42.67	64.67	57.67	21.45	NS	NS	NS
Bacteria	30 days	29.30	14.84	28.81	22.32	11.79	43322.26	NS	NS	NS
	60 days	20.26	9.10	22.94	17.29	8.05	31202.71	NS	NS	NS
	90 days	39.60	25.86	34.34	32.26	5.57	46491.72	NS	NS	NS
Fungi	30 days	6.73^{b}	17.47^{ab}	19.05^{ab}	48.93^{a}	7.36^{b}	4882.56	NS	*	*
	60 days	10.47	8.80	12.18	41.53	5.37	4832.46	NS	NS	NS
	90 days	15.70	10.62	24.60	13.76	15.99	2374.28	NS	NS	NS

[a,b,c]Means with different superscript along the raw differ significantly ($p<0.05$)

[1] 5HNa-5 g/kg diet sodium humate inclusion, 7.5HNa-7.5 g/kg diet sodium humate inclusion, 10HNa-10 g/kg diet sodium humate inclusion, 12.5HNa-12.5 g/kg diet sodium humate inclusion;

[2]L-linear, Q-quadratic, C-cubic

urease activity [29]. Ruminal NH_3-N is produced by the metabolism of proteins, peptides, amino acids, urea, nitrates and other non-protein nitrogen compounds. Inhibition of protein or peptide degradation and amino acid deamination could result in lower NH_3-N concentrations [30, 31]. The non-significant effect of sodium humate of ammonia nitrogen at 90 days feeding could be because there was more time for proteolysis and amino acid deamination to occur during the experimental period. On a contrary, under *in vitro* condition, Sheng et al. [20] observed a reduction in rumen ammonia nitrogen. On the other hand, Terry et al. [14] did not observe significant differences in rumen ammonia nitrogen of heifers on humic acid diet. The concentration of humic acid used as well as specie of animal and management system could have caused the differences in the concentration of ammonia nitrogen between this current study and that of Terry et al. [14]. Non-significant volatile fatty acids, acetate, propionate, butyrate and protozoa obtained in this study are consistent with that of Terry et al. [14]. However, Varadyova et al. [15] and Sheng et al. [20] observed a decrease in total volatile fatty acids with varying doses of humic acid *in vitro*. El-Zaiat et al. [27] also observed reduction in acetate and propionate but not total short chain fatty acid and butyrate in goats. The conditions under which these experiments were carried out would have accounted for those discrepancies in total volatile fatty acid with the current study. The cubic response observed for fungi count in this study at 30 days of sodium humate supplementation is an indication that at higher doses may not affect rumen fungi count. The action of sodium humate to increase fungi count has immense potential for boosting digestive performance in the rumen and ultimately higher animal production response. It has been reported that fungi are found attached mainly with the lignified tissues that remain in the rumen for extended periods and maintain highest number in animals receiving high-fibre diets but lack in rumen of animals receiving leafy forage, due to the shorter retention period of such feedstuffs [32]. The absorption ability of humic acids, which result in slower passage of gut content and prolonged digestion, may have been responsible for such increase in fungi count. This action of sodium humate to increase fungi count did not persist at 90 days of study may be that the forage at the time was not lignified because of regeneration from grazed forages. The no changes in protozoa count observed in this study are in agreement with the report of Galip et al. [11] in rams on humic acid diets. Protozoa counts however, increased in lamb on varying levels of humic acids [33]. As also observed in this study, there was no effect of humic acids on bacterial count of beef heifers fed a barley silage-based diet containing increasing concentrations of humic substances [14].

Conclusion

Utilization of sodium humate in the diet of semi-intensively managed WAD goats stabilized rumen pH and concentration of ammonia nitrogen reduced at higher levels used in this study. Sodium humate addition showed potential to increase fungi count but not beyond 10 g/kg diet inclusion. For animals grazing on lignified forages, this increase in fungi count could help in enhancing utilization of the nutrients in the forages.

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Ethical consideration

The authors confirm that ethical and welfare policies guiding the use of the animals for the research were adhered to and appropriate approval from ethical review committee of the University was received for the purpose of this experiment.

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Conflict of interest

All the authors of this article declare that there were no conflicts of interest throughout the period of this research.The authors have read and agreed with submissions of the findings of this research.

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