RUN -01

Diversity of Rumen Microbial Ecosystem of West African Dwarf (WAD) Rams Fed Ammonium Sulphate-Fortified Diets

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Abstract
Ammonium sulphate is a feed additive used in ruminant feeds mainly for stabilization of the intestinal flora, enhance the development of the adult rumen microflora, improve digestion and nitrogen flow towards lower digestive tract and improve meat and milk production. This study assessed the diversity of rumen microbial ecosystem of WAD rams fed ammonium sulphate-fortified diets at 0, 2.5, 5.0 and 7.5g/kg to the concentrate diet as T1, T2, T3 and T4 respectively. Sixteen WAD rams with average body weight of 12.20±0.12 kg were used and fed for 109 days, each treatment had four replicates. In a completely randomized design and differences among treatment means were examined using least significant difference, each ram was fed at 5% of their body weight at 60:40 levels of experimental diet and wilted guinea grass as basal diet. Variables measured were *S. typhimurium, E. coli,* bacteria, fungi and protozoa. The results indicated that establishment of the microbial population of diets consisting of 7.5g/kg $(NH_4)_2SO_4$ were significantly higher in bacteria and fungi than the control diet ($P<0.05$). It was concluded that fortification at 7.5g/kg $(NH_4)_2SO_4$give a better understanding of the role of rumen microbes and the establishment of the microbial population, which help to maintain the host's health and improve animal performance.

Keywords: Ecosystem, microbes, health status, WAD rams, ammonium sulphate.

Introduction

The nitrogen: sulfur ratio in the microbial proteins is 14.5:1, and a sulfur deficiency in the diet causes changes in the microbial fermentation. In this context, it is interesting to note that the utilization of non-protein nitrogen (NPN) is decreased by low levels of sulfur in the rumen fluid, implying that the microbial growth is attenuated (Cassio *et al.,* 2014). Such a deficiency also reduces the utilization of lactate by the rumen bacteria, resulting in its accumulation. Consequently, there is an appreciable reduction in the digestion of cellulose, possibly due to the reduction of the microbial growth. For this reason, sulfur improves the microbial digestion of cellulose, contributing to the synthesis of amino acids, especially methionine and cysteine.

The rumen is a complex ecosystem that harbors a wide variety of microorganisms, including bacteria, protozoa, archaea and fungi (Shaopu *et al.,* 2017). A principal function of the micro-biome is the conversion of plant materials into digestible compounds that can be used by the animal host (Mackie, 2002). This function is of tremendous importance as it allows the conversion of solar energy stored in plant fibers into food products, such as milk and meat. Additionally, as the micro-biome in the rumen undergoes long-term selection and evolution, the microbes and host form an inter-inhibitive and interdependent homeostatic relationship that has an important role in maintaining host health, improving performance, reducing environmental pollution, and ensuring food and animal product safety (Rawls *et al.,* 2004). Therefore, studies of ruminal microbes represent a key area of nutrition research in ruminants, and it is important to improve our understanding of these complex microbial populations and their interactions. Therefore, elucidating the composition of the gut bacterial community and its changes is essential to improve the health management and productivity of this important ruminant. To our knowledge, there has been no report of using ammonium sulphate-fortified diets to study developmental changes in the ruminal microbial flora in WAD rams.

Thus, this study aimed to characterize the colonization process of ruminal microbes in WAD rams using ammonium sulphate-fortified diets.

Materials and Methods

Experimental site and diets: The study was conducted in sheep unit of the Teaching and Research Farm, University of Ibadan, Nigeria. Brewers dry grain (60kg), palm kernel cake (23kg), dicalcium phosphate (1kg), oyster shell (2kg), salt (2kg), growers premix (1kg), urea (1kg) and dry cassava peel (60kg) were mixed and 0, 2.5, 5.0 and 7.5g/kg levels of ammonium sulphate were added on top of the feed. The chemical compositions of ammonium sulphate fortified diets and *Panicum maximum* fed to the experimental animals are shown in table 1.

Fifty (50) mL of rumen contents were collected from 16 rams after 109 days of feeding them with ammonium sulphate-fortified diets. All collections were performed before the morning feeding (at 08:00 a.m.). Rumen content samples were collected using suction tube. Rumen contents were squeezed through four layers of cheesecloth to remove particulate matter. Medium for culturing *S. typhimurium* and *E. coli* is McConkey agar, bacteria is nutrient agar, fungi is potato dextrose agar while the preserving solution used for protozoa was methylgreen-formalin-saline solution.

Data collected were subjected to Analysis of Variance (ANOVA) using SAS (2002).

Table 1: Chemical analysis of ammonium sulphate fortified diets and *Panicum maximum*

Parameters (%)	T1	T2	T3	T4	SEM	*P. MAXI.*
Dry Matter	94.90	94.73	94.05	94.91	0.25	38.50
Crude Protein	11.01^{c}	11.95^{c}	12.28^{b}	14.91^{a}	0.37	7.81
Ether Extract	0.62	0.70	0.63	0.78	0.01	0.70
Ash	12.04	12.30	12.19	12.80	0.02	8.94
NDF	30.35	31.71	31.67	30.06	0.02	60.00
ADF	25.20	26.58	25.96	25.34	0.01	38.00
ADL	6.16	6.18	6.12	6.14	0.03	7.19

a,b,c: Means within rows with unlike superscripts are significantly different from each other ($p<0.05$). T1: 0g/kg $(NH_4)_2SO_4$, T2: 2.5g/kg $(NH_4)_2SO_4$, T3: 5.0g/kg $(NH_4)_2SO_4$, T4: 7.5g/kg $(NH_4)_2SO_4$, SEM: Standard Error Mean,NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, *P. maxi.: Panicum maximum*

Results and Discussion

Rumen microbial diversity of WAD rams fed ammonium sulphate-fortified dietsare shown in table 2. There was significant ($p<0.05$) difference in *E. coli and S. typhimurium* of rams fed ammonium sulphate-fortified diets. The *E. coli* ranged from 2.75$\log_{10}$cfu/mL(D1) to 4.33 $\log_{10}$cfu/mL(D4). Rams fed D4 had the highest ($p<0.05$) bacterial population (4.86 $\log_{10}$cfu/mL) while those on D1 recorded the least (4.82 $\log_{10}$cfu/mL). The rams on $(NH_4)_2SO_4$ fortified diets showed an increase in bacterial population as $(NH_4)_2SO_4$ inclusion increased over those on control (D1) diet. This is suggestive of $(NH_4)_2SO_4$ stimulating the proliferation of bacterial population. Microbial load in the rumen is very important because, it is an index of the amount of microbial protein made available to the sheep daily (Kissada *et al.,* 2010). This is because sulphur is one of the important factors for fungi growth and proliferation (Gordon and Philips, 1998) that enhances microbial protein synthesis in the rumen. Low levels of sulphur supplementation in the diet containing cassava foliage could reduce microbial biomass in the rumen (Promkot *et al.,* 2007). Rams on D4 had the highest population of fungi because sulphur supplementation increased the concentration of all three microbial groups but the most dramatic increase was observed with the number of sporangial forms of rumen anaerobic fungi as shown in Table 2 which ranged from 2.53 – 2.65 $\log_{10}$cfu/mL.

Table 2: Rumen microbial diversity of WAD rams fed ammonium sulphate-fortified diets

Parameters	D1	D2	D3	D4	SEM
E. coli($\log_{10}$cfu/mL)	2.75^{d}	3.00^{c}	3.75^{b}	4.33^{a}	0.05
S. typhimurium ($\log_{10}$cfu/mL)	5.67^{d}	5.95^{c}	6.28^{b}	6.67^{a}	0.07
Bacteria ($\log_{10}$cfu/mL)	4.82^{b}	4.83^{b}	4.85^{a}	4.86^{a}	0.09
Fungi ($\log_{10}$cfu/mL)	2.53^{b}	2.57^{b}	2.64^{a}	2.65^{a}	0.75
Protozoa (/ml)	4.78	4.76	4.75	4.73	0.06

a,b,c: Means within rows with unlike superscripts are significantly different from each other ($p<0.05$). D1: 0g/kg $(NH_4)_2SO_4$, D2: 2.5g/kg $(NH_4)_2SO_4$, D3: 5.0g/kg $(NH_4)_2SO_4$, D4: 7.5g/kg $(NH_4)_2SO_4$, SEM: Standard Error Mean, *E. coli: Eschariacoli, S. typhimurium: Salmonella typhimurium*

Fungi have an additional advantage of better penetration of the lignocellulosic feeds over the cellulose-degrading bacteria because of the presence of hyphae (thread-like) structure that penetrates into the substrate. Bacteria have different fibre-degrading enzymes (cellulases, hemicellulases, xylanases, avicelases, glucosidases e.t.c.) found to be associated with rhizomycelia which enable it to degrade fibres (Paul *et al.,* 2003) after it has been exposed by fungi for degradation by bacteria. Cellulose-degrading bacteria have been extensively studied in recent decades because of their important role in supporting the host (Tomšič, *et al.,* 2007). Thus, ammonium sulphate was added to the treatments to provide adequate sulphur requirement for anaerobic fungi to maximally digest the components of dietary fibre leading to the production of energy to the animal (Okoruwa *et al.,* 2014). These results are consistent with the premise that increased rumen function due to the addition of sulphur to the diet is due to increased microbial activity.

Rams fed diet D4 had the highest ($p<0.05$) bacterial population (7.17 cfu/mL) while those on D1 recorded the least (6.67 cfu/mL). The rams on $(NH_4)_2SO_4$ fortified diets showed an increase in bacterial population as $(NH_4)_2SO_4$ inclusion increased over those on control (D1) diet. The reason for this increase can be adduced to the promotion of the growth of rumen bacteria and optimization of rumen function (i.e. ruminal ecosystem in terms of the structure of microbial populations and activities) by combinations of ammonium sulphate with the diets and this is in agreement with Akin and Benner, 1988. Bacteria can use carbohydrates as carbon skeletons for proteins in combination with ammonia (Bach *et al.,* 2005,) and also utilize sulphur (organic and inorganic) to synthesize sulphur containing amino acids (Kandylis, 1984) to produce microbial protein.

Sulphate reducing bacteria can compete against methanogens for hydrogen use by reducing methane production (Russel, 1998). Protozoa also have a negative impact on animal productivity in that the engulfment and digestion of bacteria by protozoa (Charles *et al.,* 2015) significantly lowers the flow of microbial protein leaving the rumen (Jouany and Ushida, 1999). Thus, the use of defaunation to decrease methane production from ruminants would have to be balanced against the effects on fibre and protein metabolism in the rumen. This is why ammonium sulphate-fortified diets serve as anti-methanogenic compounds because sulphur reducing bacteria compete with methanogens which led to the reduction of hydrogen as well as ciliated protozoa numbers from 5.39 (D4) to 5.95 (D1) mL.

Also, microbes in the rumen play an important role in sustaining and maintaining a balance in the rumen ecosystem. The symbiotic relationship that exists between the rumen microbes and the ruminant animal enables the animal to enjoy adequate supply of nutrients released by the microbes' activities which eventually result in the biodegradation of fibrous feedstuff abundantly available in Nigeria. Microbial population was significantly affected by ammonium sulphate fortification except for protozoa which decreased as the inclusion level of ammonium sulphate increased for all treatments. The bacterial and fungal population were higher for the ammonium sulphate-fortified diet over the control as a result of the influence of ammonium sulphate on the growth of these microbes.

Conclusion and Recommendation

In conclusion, Ammonium sulphate is a feed additive that serves as a ready source of nitrogen and sulphur, which could play an important role in enhancing the rumen microbial population of WAD rams. The inclusion of 7.5 g of ammonium sulphate into 1 kg of diet of rams is recommended to enhance microbial population to improve microbial protein synthesis in the rumen. More research is needed to better characterize these unknown bacteria and their special roles in the hosts. Also, studies on the microbial diversity of the rumen microbiota of domestic and wild ruminants are needed.

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