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Influence of Protein Source on Bacterial Population and Fermentation Products in the Rumen of Cattle

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Abstract: This study was conducted to investigate the effect of protein source (soybean meal, fish meal and leucaena leaf meal) on bacterial population and production of NH_3-N and VFA in the rumen of cattle. The bacterial counts of the collected rumen liquor were made by the general viable count method (Pour Plating Method) and production of NH_3-N and VFA was determined by Markham Distillation method. The rumenal bacterial counts were higher for soybean meal (6.70×10^6 mL^{-1}) than for other protein sources. Leucaena leaf gave the lowest bacterial counts (4.62×10^6 mL^{-1}) whereas fish meal showed the intermediate value (5.54×10^6 mL^{-1}). Concentration of NH_3-N was the highest in animals fed soybean meal followed by those fed leucaena but the lowest in animals fed fishmeal. It was concluded that among the protein sources under study, soybean meal contributed most to the bacterial population growth, ammonia and VFA production compared to those of fish meal and leucaena leaf. Among the other two protein feeds, fishmeal showed better bacterial population and ammonia production than those of leucaena.

Key words: Protein source, bacterial population, leacaene leaf, cattle

INTRODUCTION

In order to establish the level and kind of protein supplements for optimal microbial and animal response, it must be first adequately predict the degree to which protein sources are made available in the rumen from a variety of sources[1]. Because protein supplements are generally the most expensive ingredients in ruminant's rations and in most cases over-feeding or underfeeding of protein supplements hampers the physiological activity of host animal, both of which resulting an economic losses. Protein available to ruminants for intestinal digestion and absorption is a function of microbial population and with the resultant production of P^H, ammonia-N, VFA, CO_2, and other ruminal fermentable products. The quantity of ammonia-N and VFA present in the reticulo-rumen fluid is a reflection of microbial activity and of absorption or passage out of the rumen[1]. As a result, there should be a positive relationship between the growth of micro organisms in the rumen and the solubility of protein, which obviously would depend on the protein sources. With these views in mind different sources of protein supplements were studied for their influence on rumen microbial population and fermentation products.

MATERIALS AND METHODS

Animals and treatments: Three ruminally cannulated bullocks of 185 kg of average live weight were used in the experiment. Three different rations were formulated with three sources of protein, i) Soybean meal, ii) Fish meal and iii) Leucaena leaf meal for the experimental animals. The basal feeds were rice straw, dal grass, wheat bran, rice polish, and molasses. The composition of the rations is presented (Table 1).

Three animals were fed three experimental rations randomLy, so that each animal gets any one ration at a time. The overall feeding period was 17 days with 10 days adjustment period and 7 days period for collection of rumen liquor. Statistical design of the experiment followed in this study was a 3 x 3 Latin Square Design.

Collection of rumen liquor: Approximately 400 mL of rumen liquor was collected from each animal inserting a tube with gauge at its tip through the fistula and the liquor was drawn by means of pump and collected into the graded conical flask under anaerobic condition. The P^H of

Table 1: The composition of the rations under study

	Rations		
Feed ingredients (kg)	A	B	C
Rice straw	4.0	3.5	3.5
Dal grass	5.0	5.0	5.0
Rice polish	0.3	0.3	0.3
Wheat bran	0.4	0.5	0.5
Soybean meal	0.5	-	-
Fish meal	-	0.2	-
Leucaena leaf meal	-	-	1.0

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the rumen liquor was measured by a digital P^H meter immediately after collection. Then the collected rumen liquor was preserved with 10% (V/V) H_2SO_4 solution for analysis of NH_3 production. For VFA production, samples were stored immediately in deep freeze without adding any preservatives till further analysis. Simultaneously samples were also taken in a sterilized test tube for bacterial count. The bacterial counts of the collected rumen liquor were made by the general viable count method (Pour Plating Method)[2].

Determination of ruminal ammonia-N: Determination of ruminal ammonia-N in the rumen liquor was done using Markham/distillation apparatus. Water supply was turned on the condenser to ensure that the steam generator was at least half filled with distilled water. Heating mantle was switched on and was waited for continuous boiling. Boric acid indicator (5 mL) was transferred to a collection flask placed bellow the condenser. A measured quantity (0.8 g) of MgO was placed into a Kjeldahl flask was fitted to the apparatus below the sample inlet using the spring clips provided to secure it. Water supply was closed and the apparatus was allowed to steam heat for two minutes. Rumen liquor (5 mL) was placed in the reservoir around the sample inlet plunger and the plunger was lifted carefully to allow the liquid to run into the distillation flask. A quantity of 50 mL distillate was collected and titrated against 0.1 N H_2SO_4.

Calculation of ammonia-N in the rumen liquor:

1 mL 0.1 N H_2SO_4 – 1.4 mg N
1000/5 x titration in mL x 1.4 = mg NH_3 – N per litre

Determination of ruminal VFA: Determination of total volatile fatty acids in rumen liquor was also done using Markham/distillation apparatus. Water supply was turned on to the condenser to ensure that the steam generator was at least half filled with distilled water. Heating mantle was switched on and waited for continuous boiling. Collection flask was placed beneath the condenser. An aliquot of 5 mL rumen liquor was taken into a sample inlet funnel by a pipette. The plunger was lifted just enough to allow the rumen liquor to enter the still without allowing any steam to escape. This was followed immediately with 10 mL of acid MgSO4 solution allowing only about 3 mL to enter at a time so that all the rumen liquor was washed into the still and no steam or vapour escaped. Distillate of 50 mL was collected and titrated against 0.05 M NaOH as soon as it was obtained after addition of a few drops of Phenolphthalein indicator.

Calculation of total volatile fatty acids in the rumen liquor:

5 mL of rumen fluid contain,
Titration x 10 = mM VFA per litre

Analytical methods: Chemical analysis of feeds fed to the animals was done for proximate components following the methods of AOAC[3]. The data of the experiment were analysed by statistical methods using the analysis of variance[4] and the mean values were tested for difference with Least Significance Difference (LSD) using Microstast statistical package (MSTAT) in a computer.

RESULTS AND DISCUSSION

Chemical compositions of the feed ingredients of different rations are shown in (Table 2). The Table 2 showed that fish meal contained the highest level of crude protein (CP) and ash compared to other protein sources including soybean meal. Although containing high CP values, this fish meal was not high quality since it contained high percentage of ash (27%) and at the same time 45% CP was rather low value for a good quality fish meal. Leucaena a tropical legume tree fodder having reasonably high content of crude protein (23.6 %). Soybean meal and rice polish both had high fat content, 22 and 18% respectively.

Ammonia nitrogen concentrations as found in the rumen liquor of animals fed with different protein sources are presented in. The values showed that the concentration of NH_3-N (mL L^{-1}) was the highest in animals with soybean meal (128) followed by that with fish meal (116) and the lowest with leucaena (101). The statistical analysis showed that the values were significantly ($p<0.05$) different from each other.

Although protein content of fishmeal was higher than that of Soybean, the lower concentration of Nh_3-N might be due to lower degradability of fishmeal compared to that of soybean meal[5]. The lower NH_3-N concentration in fishmeal has also been reported by Mondal[6].

Table 2: Proximate components of feed items used in experimental diets

	Composition of DM (g/100 g)					
Ingredients	DM	CF	CP	Ash	EE	NFE
Soybean meal	87.0	6.5	35.4	6.1	22.1	20.3
Fish meal	87.3	3.3	44.5	27.9	8.4	3.2
Leucaena leaf meal	18.0	14.7	23.6	10.1	3.0	47.4
Dal grass	13.8	29.3	7.5	8.7	1.9	53.3
Rice straw	89.0	34.3	3.0	15.1	1.6	46.4
Wheat bran	87.9	10.2	16.7	4.5	4.7	62.8
Rice polish	89.8	17.8	14.5	14.8	18.1	3.9
Molasses	70.0	-	3.8	8.1	0.5	63.6

The total Volatile Fatty Acid (VFA) production in rumen liquor as affected by the rumen incubation of protein sources in different groups of animals are also presented in (Table 3). The volatile fatty acids production (mM/I) in animals fed on diets containing soybean meal was the highest (87.0) followed by leucaena leaf (77.3) and the fish meal being the lowest (69.3). It was found that the values of total VFA production among treatments were statistically significant ($p<0.05$). The quantity of Total Volatile Fatty Acids (TVFA) present in the reticulorumen fluids is a reflection of microbial activity and of absorption or passage out of the rumen. Following the ingestion of readily fermentable feed, microbial activity increases rapidly, resulting in an increase in VFA concentrations[1]. The result of the present study also agrees with the statement of this author. The highest concentration of TVFA in the rumen with soybean meal in the present experiment might have contributed to the nutrient supply to the microbes and consequently more bacterial population (Table 3). However, although there was higher TVFA concentration in the rumen of leucaena leaf than fish meal, the bacterial population was lower. The reason is unknown.

Ruminal P^H of different groups of animals fed different diets and incubated with different protein sources (Table 3) made it clear that there was no significant ($p>0.05$) variation among the treatments indicating that sources of protein had no much effect on pH of the rumen liquor. Lower pH value reduces rumen microorganisms specially the proteolytic bacteria, thus reducing protein degradation in the rumen[7]. The ruminal pH values of the present study are within the normal range (6.3 to 7.4) summarised by McCollough[8]. The results of the present experiment are in agreement with those reported by some workers [9,10]. This indicated that since the P^H values during the fermentation of different protein sources was within the normal range, it is assumed that microbial activity was optimum.

Ruminal bacterial population observed in this experiment given various protein sources are also shown in Table 3. Significant ($p<0.05$) differences in ruminal bacterial population were found among the treatments consisting various protein sources. Maximum numbers of rumen bacteria (6.70 x 106 mL^{-1}) were observed when cattle were fed ration containing soybean meal than those containing fish meal and leucaena leaf. Leucaena leaf meal containing ration gave the lowest value in terms of bacterial population count. The significant ($p<0.05$) differences were observed among the values of bacterial population due to differences protein source indicating primarily that the source of protein had significant effect on bacterial population, in the rumen of cattle.

The variable number of rumen bacteria observed in the experiment for different treatments may be due mainly to the nature of the diets, particularly to the sources of protein. The sources of protein also results in the variation in solubility of protein and the supply of nitrogen to the microbes. Soybean meal, during incubation In the rumen, gave rise to greater protein degradation[11] and that might have resulted in the highest microbial counts ($6.7x10^6$) per unit (mL) of rumen liquor. This result is in agreement with the findings of Mondal[6], who found the highest bacterial counts (10^6 mL) with soybean meal (6.4) compared to the other protein sources. Fish meal gave the bacterial count (5.54) lower than that of soybean meal. Zerbini *et al.*[5] also noted decreased microbial nitrogen production and efficiency in animals when fed fish meal compared to those of other concentrate proteins. The lower bacterial count due to incubation of fish meal compared to soybean meal would probably be due to the low effective crude protein degradability value as found in case of fish meal[11] and also low concentration of ammonia-N as soybean meal Table 3. The low degradation of crude protein of fish meal compared to soybean meal or other concentrate sources of protein in the rumen has also been reported by other authors[5,6] and the lowest ammonia-N concentration in the rumen for fish meal compared to other concentrate protein sources has been reported by[6]. From the result of this study it is evident that protein supplements richer in readily soluble nutrients produced greater number of total microbial population and this was supported by Warner[12]. Diets with adequate degradable protein usually results in an increase in bacterial

Table 3:Effect of dietary protein sources on bacterial population, NH_3-N concentration, total VFA production and P^H in the rumen liquor of cattle

	Sources of protein				
Parameters	Soybean meal	Fish meal	Leucaena	SED	Level of significance
Bacterial population (x 10^6/mL)	6.70	5.54	4.62	0.06	*
Ammonia-N (mg/l)	128.0	115.6	100.9	3.95	*
Total VFA production (mM/l)	87.0	69.3	77.3	1.36	*
P^H	7.0	6.8	6.9	0.06	NS

SED= Standard error of difference; * = Significant at P<0.05 level; NS= Non significant.

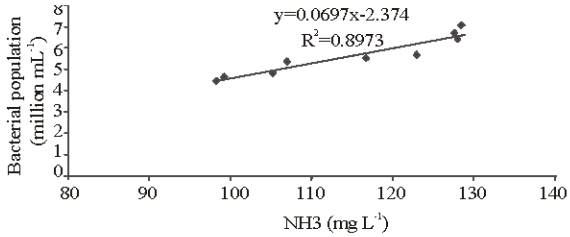

Fig .1: Relationshipbetween bacterial population and ammonia nitrogen concentrastion

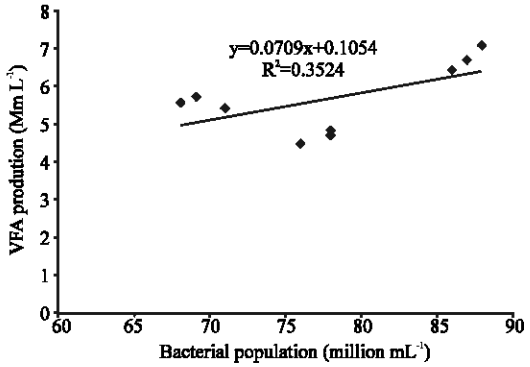

Fig .2: Relationship between bacterial population and total VFA production

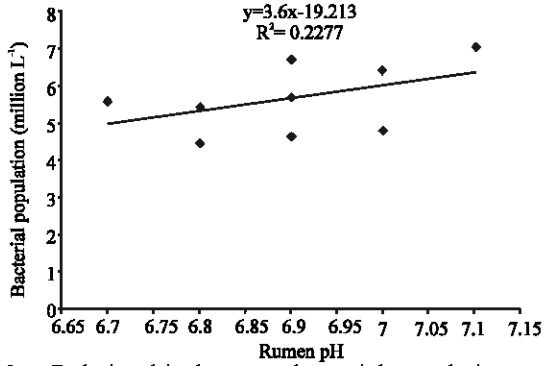

Fig .3: Relationship between bacterial population and rumen pH

numbers as compared to diets low in that type of protein[1]. Thus it is clear that the substantial differences in the bacterial population occur probably with the change in protein supplements.

(Fig . 1) shows the relationship between the bacterial population and the NH_3-N concentration in the rumen of animals fed different rations containing different protein sources. It can be seen that there was very high relationship (r^2=0.897) between these two parameters indicating that the high contribution of NH_3-N to the bacterial population. That the higher the availability of NH_3-N in the rumen enhances microbial growth has been reported by several authors[13,14].

The relationship between bacterial population and total VFA production is shown in Fig. 2. Although the relationship (r^2=0.35) was lower than that between microbial population and NH3-N, however, there were good relationship between VFA and bacterial population. Since the VFA production from protein source is not affected very much like that of protein source and NH_3-N, the relation of VFA and microbial protein production not very high.

The lowest relationship (r^2=0.22) exists between bacterial population and pH of the rumen (Figure 3). This might be due to the fact that although pH affects considerably the microbial activity, change in pH does not take place easily, so microbial population is not highly affected by it.

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