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The Relation between Ruminal Bacterial-N and Diamino Pimelic Acid ; and Bacterial Activity on Degrading This Amino Acid in Farm Ruminant Animals

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EIGHT fistulated adult male animals, two of each of cattle, buffalo, goat and sheep were fed on a concentrate mixture plus clover hay (2:3). Ruminal bacterial cells were isolated. Bacterial — N and DAP were determined. The DAP-N as percentage of bacterial — N was found to be 0.66, 0.96, 0.60 and 0.86 for cattle, buffalo, goat and sheep respectively with an average of 0.77 ± .09. The relationship between bacterial — N and DAP or DAP-N concentrations was calculated as follows :

$$Y_1 = 601.92 - 35.36\ X$$
$$Y_2 = 73.97 - 5.21\ X$$

in which Y is bacterial DAP (mg/100g DBC), Y_2 is bacterial DAP-N (mg/100g DBC), X is bacterial-N (g/100g DBC) and DBC is dry bacterial cells.

The data revealed that 11.43% of DAP could be degraded by bacteria comparing with undetected concentration with protozoa.

It has been established that rumen microorganisms play an important role in ruminant feeding. The power of digestion of ruminants is mainly due to rumen microbial enzymes. Besides, microbial protein leaving the rumen ultimately provides a source of amino acids for the host animal as the protein is digested and absorbed in the small intestine.

Various methods have been used to estimate the quantity of microbiol protein in digesta leaving the ruminant stomach. The majority of these techniques are based on determination of a single chemical marker believed to characterize the microbiol components. Diamino pimelic acid (DAP) takes advantage of the fact that it is present in the cell membrane of many types of rumen bacteria but is absent from plant materials and protozoa. The bacterial DAP was reported to be unabsorbable in the intestines (Mason

and White, 1971). However, certain strains of bacteria contain an enzyme which decarboxylates DAP to CO_2 and lysine (Dewey and Work, 1952; Dewey, 1954). The present work was mainly conducted to investigate the relationship between bacterial-N and DAP; and bacterial activity on degrading this amino acid in farm ruminant animals.

Material and Methods

Animals and feeding system

Eight fistulated adult male animals, two of each of cattle, buffalo, goat and sheep were used in the present work. The animals were fed a ration contained concentrate mixture (CM) plus clover hay (2 : 3) for two months to cover their requirements (NRC, 1971, 1968). The concentrate mixture consisted of undecorticated cotton seed cake and rice bran (1 : 1) plus 2% calcium hydroxide and 1% salt.

Preparation of bacterial cell walls

Rumen liquor (RL) samples were withdrawn from each animal through the fistula after mixing in a cylinder (500 ml capacity) before feeding. The protozoa and plant débris were first separated off by gentle centrifugation after straining through muslin and the bacteria in the supernatant liquid were collected in a sharple super centrifuge (16000 r.p.m. for 30 min). The bacterial cells in water were heated for 5 min at 100° according to the procedure described by Salton and Horne (1951). The suspensions heated at 100° were centrifuged at 3,000 r.p.m. for 20 min. The deposit of cell bodies was discarded, the supernatant fluid collected and the cell walls removed from the cell solutes by centrifugation for 10 min at 10,000 r.p.m. then the bacterial cell walls dried at 65°.

Protozoal and bacterial preparation

Rumen content samples (500 ml) were taken through the fistula before feeding. The samples were strained through muslin and put in pre-warmed 500 ml cylinder under CO_2 atmosphere, then incubated at 39° till the plant debris floated at the surface. Debris were carefully removed by using a pipe connected with

water pump. The remainder centrifuged at 5000 r.p.m. for 15 min. The supernatent contained bacterial only and the residue consisted of protozoa. The bacteria in the supernatent liquid were collected in a sharple super centrifuge (16000 r.p.m. for 30 min). The residue was reffered to as bacterial fraction and the remainder as clarified rumen liquor (CRL). The protozoa were washed several times by using warm Hungate buffer (39°) bubbled with CO_2 until it was free from plant particles and bacteria (Hungate, 1966). The bacterial preparation was obtained by adding 20 ml of clarified rumen liquor (CRL)) to the bacterial fraction + 20 ml of Hungate buffer, CO_2 was bubbled through the liquid. The protozoal preparation was obtained also by adding 20 ml of CRL and 20 ml of the same buffer to separated protozoa, CO_2 was bubbled through the liquid.

Determination of DAP degradation in vitro

Six tubes were used for each preparation in which two of them served as control. In two tubes 20 ml of the bacterial preparation + 25 ml of Hungate buffer + 2 gm of dried bacterial cell walls + 0.2 gm of starch were added.

In two other 20 ml of the protozoal preparation + 25 ml of Hungate buffer + 2 gm of dried bacterial cell walls + 0.2 gm of starch were added.

Two ml of HCl (6N) was added to the control tubes. The other tubes were incubated for 3 h at 39° followed by adding 2 ml of HCl (6N). After that these samples were dried at 65° for DAP determination. The experiment was repeated three times.

DAP and Cp determination

The determination of DAP was conducted using the method of El-Shazly and Hungate (1966) as modified by Mason and White (1971) and Zaki El-Din (1976). Nitrogen determination was carried out by the microkjeldhal method (A.O.A.C., 1975).

Results and Discussion

Table I shows that DAP concentration was different in the different species. It was highest in buffalo (326.8) and was lowest in goat (261.2). The concentration of DAP among the tested species was 297.00 ± 14.26 in average.

The bacterial-N was between 5.01 to 6.44 g/100g dried bacterial cells with an average of 5.80 ± 0.37. The concentration of DAP-N as % of bacterial-N was highest in buffalo (0.961) and was lowest in goats (0.598). These findings may suggest that the rumen of buffalo and sheep contains higher concentration of bacteria or higher proportion of gram-negative bacteria than cattle and goats.

Table 1 : Average date of DAP, DAP-N, Bacterial-N and DAP-N% of bacterial-N .

Species	DAP	DAP-N	Bacterial -N	DAP-N% of bacterial- N
	mg/100gDBC	mg/100g DBC	g/100g DBC	
Cattle	288.8	42.56	6.419	0.663
Buffalo	326.8	48.16	5.014	0.961
Goat	261.2	38.49	6.438	0.598
Sheep	311.2	45.86	5.309	0.864
Average	297.0	43.77	5.8	0.772
CV	9.6	9.60	12.79	21.99

Conc. = Concentration, DBC = Dry bacterial cells, CV = Coefficient of variation, DAP - N = (28/190) (100) = 14.7368.

The negative relationship between bacterial-N and DAP or DAP (Table 2) shows that as the content of bacterial cells of N increased as DAP or DAP-N concentration decreased. This may suggest that the proportion of DAP decreased as the size of bacterial cells increased. The Wark of Czerkowski (1974) support this view. He found that the proportion of DAP was low in large bacteria when compared to the small ones. Such a finding may suggest that bacterial DAP is presented in a certain limit as shown from the negative correlation (-0.92) presented in Table

The rumen bacteria concentration of DAP-N may assist greatly in predicting bacterial-N if the relationship between bacterial-N and DAP or DAP-N is constant. This relationship is, however, hot constant according to the difference in the type of ration. While DAP-N as % of bacterial-N was 0.77 in the present study, it was 0.64% when feeding on wheaten hay (Weller, 1958). Thus, effect of the type of diets on this relationship using larger number of animals and species still waranted.

Table (2) : The relationship between bacterial-N (X) and DAP or DAP-N.

X	Y	n	r	r^2	the relationship
Bacterial - N (g/100g DBC)	DAP conc (mg/100g DBC)	6	-.92 xx	0.85	Y= 501.92 - 35.36 X
	DAP-N conc. (mg/100g DBC)	6	-.92 xx	0.85	Y= 73.97 - 5.21 X

n = Number of observations, r = Correlation Coefficient, r^2 = Coefficient of determination.

xx = P < 01.

The rate of DAP degradation of bacteria and protozoa (Table 3) showed that bacteria can degrade the DAP and utilize it. Dewey and Work (1952) reported that *E. coli* contains an enzyme which decarboxylates natural DAP to CO_2 plus lysine. They showed that this enzyme is DAP decarboxylase. Furthermore, Dewey (1954) showed that this enzyme is widely distributed among 58 coli aerogenes organisms. The rate of bacterial degradation was about 11.45% (Table 3). This rate of degradation shows that bacteria can degrade DAP at a low rate. On the other hand, it seems that protozoa had no DAP degrading enzymes and only the bacteria contain the enzymes which can degrade the DAP.

Table 3: Average data of the rate of DAP degradation.

Item	Incubation time	DAP Conc.	Degradation rate	
	(h)	mg/100gRC	mg/100gRC/h	%
Bacteria	0	52.50		
	3	46.50	2.0	11.43
Protozoa	0	39.90		
	3	40.00	0.0	0.00

RC : rumen contents.

As a conclusion it seems that DAP-N as % of bacterial-N differ according to animal species and to the presented diet to a greater extent. However, the highly negative correlation between bacterial-N and DAP or DAP-N can be used satisfactory if equations were derived from wide range of diets. Also, it seems from the date of the present study that bacterial DAP is presented in a certain limit specially as shown from the negative correlation in Table 2.

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العلاقة بين النيتروجين البكتيرى وحمض البيمليك ثنـــائى وكذلك النشاط البكتيرى فى تحليل هذاالحمض فى المجترات

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غذيت ثمانية ذكور (اثنين من كل من الابقار والجاموس والماعز والاغنام) على مخلوط مركز ودريس برسيم بنسبة ٣:٢ وعزلت بكتريا الكرش وقدر كل من النيتروجين البكتيرى وحمض البيمليك ثنائى الامين . ووجد ان النسبة المئوية لنيتروجين حمض البيمليك ثنائى الامين كانت ٠ر٦٦ ، ٠ر٩٦، ٠ر٨٦ للابقار والجاموس والماعز والاغنـــام على التوالى بمتوســـط قدره ٠ر٧٧ + ٠ر٠٩ وحسبت العلاقة ما بين حمض البيمليك ثنـــائى الامين (Y_1) أو نيتروجين هذا الحمض (Y_2) بالمليجرام/١٠٠جم خلايا بكتيرية جافة والنيتروجين البكتيرى (x) بالجرام لكل ١٠٠ جم خلايا بكتيرية جافة كما يلى :

$$Y_1 = 502.92 - 35.36\,X$$
$$Y_2 = 73.97 - 5.21\,X$$

واوضحت البيانات ان البكتيريا تســـتطيع تحليل ٤٣ر١١٪ من حمض البيمليك ثنائى الامين بالمقارنة بالبروتوزوا والتى وجد انها لا تقوم بتحليل هذا الحمض .