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Effect of isoacids on some rumen enzymes

A. Moharrery[1]

Animal Science Department, Agricultural College, Shahrekord University, Iran

ABSTRACT

Four total mixed rations prepared from finger millet straw as a roughage (48%) and mixed concentrate (52%), supplemented with 1 or 1.5% isoacids and protein (groundnut cake, 5% crude protein more than control) were given in a change-over experiment to sheep. Biochemical activities were estimated for enzymes e.g., urease, cellulase, protease, and amylase, in various fractions of rumen fluid. Rumen samples were fractionated by centrifugation in strained rumen fluid without protozoa (SRFWP), cell free rumen fluid (CFRF) and enzymes associated with the bacterial cell (EABC). Samples of SRFWP and EABC contained higher enzyme activity than CFRF. These values showed very close cooperative action between proteolytic and amylolytic bacteria under the experimental condition, or perhaps presence of some species of bacteria with both activities. Results showed isoacid and crude protein enhanced microbial function ($P<0.05$) and this can change the pattern of enzymes in the rumen of sheep.

KEY WORDS: sheep, isoacids, rumen, enzyme

INTRODUCTION

Any biotechnological developments will undoubtedly have to target the improvement of efficiency of ruminants under practical conditions pertaining particularly to small farms. One of the major ways by which any technology may significantly improve livestock production is manipulating the fermentation, gastric and post-gastric digestive process to extract more and a better balance of nutrients for the animal from the basal feed. Considerable progress has been made toward understanding quantitative relationships among the chemical composition of ruminant feeds, dynamic aspects of digestion in the rumen, products from digestion absorbed by the ruminant and most important, how these can be manipulated to improve animal productivity. Branched-chain fatty acids (ioacids), isobutyric (i-C_4), 2-methylbutyric (2 Me-C_4) and isovaleric (i-C_5) and

[1] Corresponding author: e-mail: moharrery@yahoo.com

the straight-chain valeric acid (n-C_5) are naturally produced in the digestive tract of the ruminant (Andries et al., 1987) and rumen microorganisms have ability of converting isoacids to amino acids. In this regard, the objectives of the present experiment were to study effects of isoacids and protein on enzyme pattern and correlation between them in rumen.

MATERIAL AND METHODS

Preparation of mixed rumen bacteria, from the ruminal content, taken by a suction pump 3-4 h after morning feeding the sheep through a stomach tube. The rumen contents were mixed, and squeezed through four-layered gauze. The squeezed fluid was centrifuged once at 1000 × g for 10 min. The supernatant fluid was carefully decanted and fractioned into three parts. One part was stored at -196˚C for future analysis and named strained rumen fluid without protozoa) (SRFWP), another part (25 ml) was then centrifuged again (26000 × g, 15 min) and supernatant fluid was carefully decanted and stored at -196˚C as a sample without any germ, and named cell free rumen fluid (CFRF). Pellet was resuspended in buffer solution and sonically-disrupted cells were centrifuged at 40,000 × g for 10 min. The clear supernatant was kept at -196˚C and named enzyme associated with the bacterial cell (EABC). Measurements of enzymes were done according to Moharrery and Das (2001).

RESULTS AND DISCUSSION

The effect of different treatment on enzyme activity in the three fractions is presented in Table 1. Any treatment compare with control, increased urease activity. There was no significant difference between, isoacids and protein treatment ($P>0.05$). These treatments have 45, 67 and 69% more urease activity than control, respectively ($P<0.05$). Greatest cellulase activity was found after isoacid treatment ($P<0.05$). An increase of 19% in cellulase activity compared to control showed isoacids as a complex component, which can encourage cellulolytic bacteria. Other treatments did not give any significant difference to compare with control diet. Also no difference can be detected statistically between treatments for protease activities ($P>0.05$). In three fractions of rumen fluid, it is best to use the coefficient of determination (r^2) to explain the degree of association between two variables. In this regard, r^2 for protease with amylase is 0.373. This correlation signifies that 37.3% of the total variation in protease activity can be explained by the relationship between protease activity with the amount of amylase.

Table 1. Enzyme activity in three fractions of rumen fluid: rumen fluid without protozoa (SRFWP), cell free rumen fluid (CFRF) and enzymes associated with the bacterial cell (EABC)

	Diets				SE
	control	1% isoacids	1.5% isoacids	protein	
SRFWP					
urease	6.01	8.71	9.30	10.14	1.345
cellulase	738.5^{b}	874.6^{a}	656.6^{b}	704.2^{b}	39.38
protease	0.201^{a}	0.186^{a}	0.113^{b}	0.163^{ab}	0.0200
amylase	174.6^{b}	161.6^{b}	242.3^{a}	90.1^{c}	18.88
CFRF					
urease	7.03^{b}	9.02^{b}	6.60^{b}	16.60^{a}	2.164
cellulase	162.2^{b}	224.2^{b}	348.6^{a}	249.0^{b}	28.97
protease	0.090	0.085	0.202	0.164	0.0560
amylase	60.1	63.1	69.2	44.3	10.96
EABC					
urease	9.28^{ab}	5.03^{b}	17.89^{a}	14.28^{ab}	3.430
cellulase	405.5	563.0	343.5	706.1	159.35
protease	0.220	0.204	0.281	0.246	0.0356
amylase	208.7	102.4	217.2	290.1	58.62

urease (μg ammonia-N/min/ml), cellulase (μg gluc./h/ml), protease (Unit/ml), amylase (μg gluc./min/ml)

means with the same letter in each row are not significantly different ($P<0.05$)

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