C33.33

Function verification of ruminally protective layer of protein tablets

J. Třináctý[*1], M. Richter[1], P. Doležal[2], G. Chládek[2], J. Szakács[3].

[1]*Research Institute for Cattle Breeding, Ltd., Dep. Pohořelice, Vídeňská 699, 691 23 Pohořelice, Czech Republic,*
[2]*Mendel University of Agriculture and Forestry Brno, 613 00, Czech Republic,*
[3]*Liaharenský podnik Nitra, company, Slovak Republic*

- *Corresponding autor:* trinacty@vuvz.cz

Abstract

The ruminal degradability of soluble nitrogen fractions is high (i. e. more than 80%) and the application of rumen non-protected amino acids and protein concentrates would be uneconomical. The ruminal protection can assure that these important nutritional substances will be transported into the small intestine with only minimal losses.

The aim of the experiment was to confirm the functionality of layer protecting the protein tablets against the rumen activity using in vivo method. For this purpose as a marker amino acids (Met, Lys, His) supplementing the amino acid composition of soya protein added to tablets were used.

This study was supported by NAZV project No. 1B44037

Material and Methods

Animals and procedures

Three lactating Holstein cows weighing on average 523 kg were fitted with ruminal and duodenal cannulas. The experiment was divided into 4 periods. Each period (14 d) consisted of 10 d preliminary period and a 4 d experimental period. In the first period one cow received the tablets (T group) and the other two received the powder (C group – control) with the same composition. Cows were fed individually twice daily (7.00 and 16.35 h) *ad libitum* a mixed diet based on a corn silage (54.7%), alfalfa hay (15.0%), supplemental mixture (30.3%) and tablets or powder (as supplement).

The supplement consisted of purified soya protein concentrate HP 300 enriched with amino acids (Met, Lys, His) in amounts calculated with regard to requirements of experimental animals (Rulquin et al., 2001a, 2001b). The supplement consisted of 93.0 % HP 300, 2.4 % Met, 1.6 % Lys and 3.0 % His. One half of this mixture was tableted (T) while the other was used as a powder (C). Lenticular tablets with the diameter of 6.5 mm were coated with a polymeric material on a base of vinyl-pyridine/styrene copolymer (Ardaillon et al., 1989). During the whole experimental period paper boluses containing either tablets or powder plus 7.5 g of chromium oxide which was used as a marker of digesta passage were placed into the rumen bottom of each dairy cow through the ruminal cannula twice daily before feeding.

Duodenal chymus (500 ml) was sampled from each animal in six-hour intervals during the whole four-day experimental period starting on 7.00 am of the first day. On each

subsequent day the time of sampling was postponed by 1.5 hour so that the four-day experimental period represented a set of chymus samples obtained during the day in 1.5 hour intervals (Schwab et al., 1992). The obtained samples were immediately frozen to –20° C.

Feed samples were taken on the third day of each experimental period and the feed refusals were collected and weighed prior to each feeding during the experimental period. These refusals were also kept frozen at –20° C. After the end of the experimental period samples collected from each dairy cow were pooled, homogenised and used for the preparation of a representative sample.

Processing and analyses of chymus and feed samples

Chymus samples obtained within a four-day experimental period were thawed and pooled for each dairy cow and each period. They were continuously stirred and used for the recovery of four average samples (500 ml). Chymus samples were lyophilised while feed and feed refusals samples were dried at 55 °C for 48 hours. After the equilibration of water content to laboratory humidity all samples were ground and sifted through a 1-mm screen. In feed and feed refusals samples the following parameters were estimated according to AOAC 1984: dry matter (DM), ash (A), crude protein (CP), fat and crude fibre (CF). NDF (with α-amylase) and ADF were estimated according to Van Soest et al. (1991). Content of Cr in samples of duodenal chymus were estimated according to Williams et al. (1962). In these samples, ammonia nitrogen was estimated in a water eluate (2g/100ml of distilled water) using a gas electrode (manufacturer Radelkis, Hungary). Total soluble nitrogen and soluble nitrogen after trichloroacetic acid precipitation were estimated using a modified method described by Licitra et al. (1996): buffer was replaced by distilled water and the samples were centrifugated at 27,000 g and 4°C due to an impaired filterability; nitrogen was estimated in the supernatant using the Kjedahl method. In these samples, free amino acids (FAA) were estimated as well using the following method: 2 g of the sample were shaked for 30 min in 10 ml of distilled water with a supplement of 5 ml of 10% sulphosalicylic acid. After the filtration the turbidity was removed by centrifugation at 10,000 g for 10 min. Detectable free amino acids were estimated in an automatic analyser AAA 400 (Ingos, CR) using a Li citrate buffer system.

Statistical analysis

Statistical analysis of obtained data was performed using the GLM procedure from the statistic software SYSTAT 11.00.01. (USA). The following equation was used as a model for the comparison of concentrations and flows of nutrients in duodenum:

$Yij = \mu + Ti + Cj + TiCj + \varepsilon ij$,

where: μ= total average, Ti = effect of the experimental factors (i = 2), Cj = effect of the dairy cow (j = 3) and εij = residual error. Unbalanced numbers of animals in each period (1 v. 2) induced lost of degrees of freedom. From this reason the effect of period was propagated to the residual error.

Results

The DM intake was significantly higher ($P<0.05$) for cows receiving rumen-protected tablets (T, 16.01 kg/d) than in the control group (C, 15.47 kg/d). Increased DM intake was followed by increased consumption of other nutrients as presented in the previous article (Třináctý et al., 2006). Average milk yield of the T group was higher (17.75 kg/d, $P<0.05$) than that of the control group (16.82 kg/d). The intake of individual components from tablets (T) or powder (C) was identical in both groups.

Because of the significant difference in DMI between experimental groups values of duodenal flows were converted according to DMI. Results are presented in Table 1. The DM flow through duodenum did not differ significantly ($P>0.05$) between treatments. Similarly, flows of all nitrogen fractions: total nitrogen (TN), total non-ammonia nitrogen (TNAN),

soluble non-ammonia nitrogen (SNAN),), soluble protein and long-chain peptides nitrogen (SPLPN) and soluble short-chain peptides and free amino acids nitrogen (SSPFAAN) were not affected by the treatment ($P>0.05$) nevertheless duodenal flow of mentioned parameters tended to be higher when tablets (T) were given.

Flow of Met, Lys and His used for the supplementation of soya protein was significantly higher ($P<0.01$) in the T group than in the C one. After usage of the rumen-protected tablets duodenal flow of Lys increased 2.8 times, Met 4.0 times and His 4.3 times in comparison with non-protected powder form. Also flow of other essential amino acid (EAA) Arg in the T group was significantly higher ($P<0.05$) than in the C one. This result is correlated with a relatively high content of this amino acid in soybean protein (Gonzáles et al., 2000). Flows of remaining EAA (Ile, Leu and Phe), NEAA and endogenous AA (GABA, Gly, Cysteic acid, Ornithine, Tau and Tyr) did not differ significantly ($P>0.05$) between treatments but tended to be higher in the T group in comparison with the control.

Conclusions

This experiment confirmed the functionality of ruminally protected tablets containing amino-acid-supplemented soya protein by significant increase of duodenal flow of free amino acids: Met, Lys, His and Arg.

References

AOAC, 1984. Official Methods of Analysis, Association of Official Analytical Chemists, 14th Edition. Washington, DC

Ardaillon P., Antant P., Bourrain P., Cartillier A., 1989. Compositions for coating feedstuff additives thus coated. United States Patent, US 4,877,621

Gonzáles J., Rodríguez C. A., Centeno C., Lamnari F., 2000. Rumen effective degradability of amino acids from soybean meal corrected for microbial contamination. Rep. Nutr. Dev. 40, 579-586

Licitra G., Hernandez T. M., Van Soest P. J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Tech. 57, 347-358

Rulquin H., Verite R., Guinard-Flament J., 2001a. Tables des valeurs AADI des aliments des ruminants, INRA Prod. Anim. 14, Suppl. 16 p

Rulquin H., Verite R., Guinard-Flament J., Pisulewski P. M., 2001b. Acides aminés digestibles dans l´intestin. Origines des variations chez les ruminants et répercussions sur les protéins du lait, INRA Prod. Anim. 14, 201-210

Schwab C. G., Bozak C. K., Whitehouse N. L., Olson V. M., 1992. Amino acid limitation and flow to the duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. J. Dairy Sci. 75, 3486-3502

Třináctý J., Křížová L., Hadrová S., Hanuš O., Janštová B., Vorlová L., Dračková M., 2006. Effect of rumen-protected supplement with three amino acids on milk yield, composition and fatty acids profile in dairy cows. J. Anim. Feed Sci. 15, 3-15

Van Soest P. J., Robertson J. B., Lewis B. A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597

Williams C. H., David D. J., Iismaa, O., 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J.Agric. Sci. Cam. 59, 381-385

Table 1. Duodenal flows of DM, nitrogen fractions and free amino acids converted according to dry matter intake

Item	Tablets		Control		P[1]
	Mean	SEM	Mean	SEM	
Total nitrogen and soluble nitrogen fractions (g / day/kg DMI)					
DM	571,1	16,8	566,0	35,0	NS
TN[2]	22,0	0,8	20,8	1,0	NS
TNAN[2]	21,1	0,8	19,6	1,0	NS
SNAN[2]	7,9	0,3	7,4	0,4	NS
SPLPN[2]	0,3	0,1	0,3	0,2	NS
SSPFAAN[2]	7,6	0,36	7,1	0,4	NS
Free amino acids (mg / day/kg DMI)					
EAA					
Arg	24,4	7,7	9,9	1,7	*
His	143,7	23,5	33,5	10,4	**
Ile	7,1	1,4	4,6	0,8	NS
Leu	64,9	14,0	46,6	7,1	NS
Lys	142,2	22,4	50,0	7,9	**
Met	89,0	14,0	22,4	5,7	**
Phe	90,0	23,1	69,4	10,0	NS
NEAA and endogenous AA					
GABA	110	32	95	14	NS
Gly	1166	356	761	136	NS
Cysteic acid	80	27	60	8	NS
Ornithine	24	6	18	2	NS
Taurine	221	76	103	24	NS
Tyr	91	22	71	12	NS

[1] means differ significantly *P<0.05, ** P<0.01; non-significantly NS

[2] total nitrogen (TN), total non-ammonia nitrogen (TNAN), soluble non-ammonia nitrogen (SNAN),), soluble protein and long-chain peptides nitrogen (SPLPN), soluble short-chain peptides and free amino acids nitrogen (SSPFAAN)