THE EFFECTS OF A NON STARCH POLYSACCHARIDASE ENZYME PREPARATION FROM THERMOMYCES LANUGINOSUS ON THE RUMINAL VOLATILE FATTY ACID PRODUCTION, ENERGY AND PROTEIN METABOLISM AND MILK YIELD OF DAIRY CATTLE

E. Brydl[1], P. Rafai[1*], V. Jurkovich[1], L. Könyves[1], Mrs. L. Tegzes[1], J. Kutasi[2], Á. Bata[2], G. Nagy[3], J. Bartyik[4], A. Fülöp[4]
E-mail: ebrydl@univet.hu

[1]Szent István University, Faculty of Veterinary Medicine, Department of Animal Hygiene, Herd Health and Veterinary Ethology and
[3]Department of Pharmacology and Toxicology, H-1078 Budapest, István u.2., Hungary,
[2]Dr Bata Research and Development Ltd (LLC), H-2364 Ócsa, Pesti úti major, Hungary,
[4]Enying Agricultural Share-holders Co., H-8130 Enying, Kossuth L. u. 42., Hungary

Abstract

In the present study the effects of an enzyme preparation (Rumino-Zyme) high in xylanase activity were studied on the ruminal volatile fatty acid (VFA) production, parameters of energy and protein metabolism, milk yield, feed conversion rate (FCR) and body condition score of high yielding dairy cows. The lignolytic enzyme preparation applied in the present experiment and fed to dairy cows at 34 g/day dosage increased the VFA concentration in the rumen from about 32 days after calving and onward. Increased VFA production has been followed by about 5 to 10% increase in the milk production and almost 0.1% increase in the butterfat production. Increased VFA production produced more balanced energy metabolism in the experimental cows as indicated by the lower incidence rate of hyperketonaemia, and lower aceto-acetic and Non Esterified Fatty Acid (NEFA) concentration in the blood of the experimental cows. Aspartate Amino-transferase (AST) activity was tendentiously higher in the control group and the proportion of control cows that had AST activity higher than 100 U/l was also higher in the control group. Both control and experimental cows showed balanced protein and acid-base metabolism throughout the experiment. Enhanced VFA production contributed to the betterment of energy balance in the experimental cows with a resultant improvement of feed intake and feed utilisation. Due to more balanced energy metabolism post-parturient weight loss of the treatment cows was lessened.

Keywords: Dairy cattle, Thermomyces lanuginosus, Rumino-Zyme, milk production, body condition, feed conversion rate.

Abbreviation key: Volatile Fatty Acids (VFAs), aceto-acetic acid, Non Esterified Fatty Acid (NEFA), aspartate amino-transferase (AST), Net Acid-Base Excretion (NABE).

INTRODUCTION

External Non Starch Polysaccharidase (NSP) enzymes are used in two ways. Firstly: enzyme pre-treatment of roughages and forages is regarded very promising. Nakashima and Orskov (1989), Beauchemin et al. (1995), Stokes (1992), Chamberlain and Robertson (1992), Kung et al. (2000), Stokes (1992).

Secondly: direct feeding of enzymes in the daily ration is expected to aid the digestive processes in the stomach(s) and intestines, presuming the enzymes preserve their polysaccharidase activity in the gut. Chesson (1994) was sceptic about the beneficial effects of saccharidase enzyme preparations in feeding of ruminants, because these enzymes may become inactivated in the rumen suspected. Later Hristov et al. (1998) attested the stability of these enzymes in the ruminal environment and proved their quick inactivation at the low pH of the abomasum.

Direct feeding of enzymes to cows increased the milk production by about 5 to 10% at the beginning of the lactation (Kung et al., 1997; Nussio et al., 1997) and in other experiments (Lewis et al., 1999; Schingoethe et al., 1999.) in the middle of the lactation. Schingoethe et al. (1999) reported an increment in the butterfat and milk protein production of the treatment cows, however, authors failed to explain the background of the improvement. Other experiments (Lewis et al., 1996; Howes et al., 1998; Yang et al., 1999) have shown that saccharidases increased the ruminal concentration of volatile fatty acids (VFAs). Direct feeding of these enzymes increased the microbial protein synthesis and improved ruminal digestibility of the fibre fraction (Yang et al., 1999; Beauchemin et al., 2000). In cows with positive energy balance enzymatic treatment proved inefficient (Beauchemin et al., 2000).

The positive and in some respect controversial data of the relevant literature have prompted us to study the effects of an enzyme preparation (Rumino-Zyme) high in xylanase activity on the ruminal volatile fatty acid production, on fat and carbohydrate metabolism, and energy balance and on milk production of dairy cows.

MATERIALS AND METHODS

The enzyme preparation: Lignolytic enzymes are produced industrially by fungal cultures (Schülien et al., 1992; Van de Mierop and Ghesquiere, 1998; Chamberlain and Robertson, 1994; Sanchez et al., 1996). In our laboratory a thermophilic fungus, Thermomyces lanuginosus, known to produce cellulase free extracts high in xylanase and low in β-xylosidase, β-glucosidase and α-arabinosidase activity (Purkarthofer and Steiner, 1995; Bennett et al., 1998.) was used to produce an enzyme preparation as reported elsewhere (Kutasi et al., 2001). The product (Rumino-Zyme) is a light-brown granulate (particle size: 400-500 μm) of 90% dry matter (DM) content, which contains thermal resistant endoxylanase from the fungus Themomyces lanuginosus. IUB ranking of the enzyme is: endo-1.4-ßxylanase, which preserves its activity within the range of pH 4.5-8.0 and 30-40 °C. Shelf life at 20 °C is longer than 6 months. Enzyme activity of the product is 2 000 IU*/g. The preparation hydrolyses xylans and arabino-xylans into mono-, bi-, tri- and oligo-saccharides.

Place and time of the study: The experiment was carried out at a loose housing dairy cattle farm of 2 000 Holstein Friesian cows between October 1999 and February 2000.

Animals and diets: By pairing on basis of equal production and parity, two hundred ear tagged Holstein Friesian cows of 2nd and 3rd lactation was assembled into an experimental and a control group of equal size. Housing and feeding regime of the experimental and control cows was identical with the exception that daily ration of the experimental cows contained 34 g enzyme preparation (Rumino-Zyme) in 1 kg Protavit Hepar super concentrate from about 3 weeks prior to expected calving till the 110th day of lactation.

IU: one unit of xylanase activity expressed as μmol of reducing /xylose equivalent/ sugar released in one min

Data recording and samplings: Of the 100 experimental and control cows, 10 cows in each group were designated for taking ruminal fluid, blood and urine samples by about two weeks intervals from the beginning of the experiment (9±4.6 days before the expected parturition) till its end (107±4.6 days after parturition).

VFA concentration of the ruminal fluid samples was measured gas chromatographically.

Fat- and carbohydrate-metabolism and -balance were monitored by determination of the glucose, acetic acid and NEFA concentrations in the blood samples. Data of the acid-base balance was also considered. Subclinical fat mobilisation syndrome was studied on basis of the NEFA concentrations and AST activity of blood samples. Occurrence of hyperketonaemia was judged on basis of the aceto-acetic acid concentration of the blood samples.

Glucose concentration of the blood samples was determined by the method described by Trinder (1969). Urea was measured by an enzymatic method (Tietz, 1987). Activity of AST was estimated by a kinetic method (Bergmeyer et al, 1976) suggested by IFCC (International Federation of Clinical Chemistry). These examinations were carried out by an Autohumalyser 900S Plus clinical-chemical analyser (Human GmbH, Germany). The NEFA and the aceto-acetic acid concentration of the blood samples was measured by the method of Noma et al. (1973) and Walker (1954), respectively by using a Unicam Helios Gamma photometer equipped with automatic samplers (Unicam Ltd., UK).

Acid-base balance was studied by determination of the urinary pH and net acid-base excretion (NABE, Kutas, 1965).

Milk yield of the experimental and control cows was recorded by milking and computed for daily production by a Pro Vantage^{TM} 2050 Integrated Management System adopted to the Bou-Matic milking system. Butter-fat concentration was measured once a month by the laboratory of the Institute of Herd Recording (Gödöllő, Hungary). Feed intake of the cows was measured per feedings by the weighing-instrument of a Seko-Self 500/145 L feed mixer-wagon. Data of feed distribution was recorded and processed by MC 2 000 (V 1.147) software, interfacing between the scale and the computer.

Body condition of the cows was scored at the time of taking ruminal fluid samples by using a 1 to 5 grade scoring system according to Mulvany (1977).

Statistical analysis: Milk production of the control and experimental cows was recorded from the time of parturition up to the 110th day of lactation. The first and consecutive 10-day's milk production of each cow was averaged and the between group differences of these ten-day means (±SD) of the experimental and control cows were studied by the Student's t-test (Microsoft Excell 98) for significance. This same test was used for analysing the data of ruminal fluid and other biological samples.

RESULTS AND DISCUSSION

VFA concentration of the ruminal fluid: Ruminal acetic-acid concentrations of the groups were almost identical prior to and right after calving and no statistically significant difference was found between the groups in the later phase of the experiment till the 6th and 7th samplings at day 75 and 107 post partum, respectively, where the experimental cows produced significantly more acetic acid than the controls.

The average propionic-acid concentration of the experimental cows proved inferior to the controls at the 1st sampling and in the first month of lactation there was no major difference be-

tween control and experimental cows. From the 5th samplings onward, however, the propionic-acid concentration showed steady increase in the experimental animals over the controls.

The concentration of n-butyric-acid in the ruminal fluid samples of the experimental and control cows was almost identical prior to calving (1st sampling), then in the next two samplings this parameter of the experimental animals lagged behind those of the controls. In the 2nd part of the experiment (from the 4th samplings onward) the experimental cows produced more n-butyric-acid in the rumen in comparison with the controls.

The concentrations of the total VFAs followed the pattern of the three organic acids discussed above. There was a higher VFA concentration in the experimental cows in the 2nd half of the experiment.

Energy and protein metabolism: Glucose concentrations of the blood samples of the control and experimental cows ranged between 2.6-3.3 and 2.6-3.5 mmol/l, respectively throughout the examination with no statistically significant differences between control and treatment cows.

The serum aceto-acetic acid concentrations of non-treated cows were higher than those in the experimental cows throughout the experiment. The difference between averages of the groups proved significant on days 22, 75 and 107 post partum. It is accepted that aceto-acetic concentration higher than 0.1 mmol/l indicates the presence of hyperketonaemia (subclinical ketosis) (Brydl, 1999, Brydl et al., 2000, Radostits et al., 2000). The group average of the control cows on day 22 post partum was higher than 0.1 mmol/l and the difference between the experimental and control cows was statistically significant ($P<0.01$). Further to this, analysis of the data revealed 11.1, 16.7, 14.3 and 12.5% incidence rate of subclinical ketosis in the control group on day 10, 22, 32, and 58, respectively. The incidence of hyperketonaemia in the experimental group was considerably less with 8.3 and 11.1% on day 10 and 22 after calving. There was not hyperketonaemia on day 32 and 58 in this group.

The NEFA concentration in the plasma of the control cows on day 10 after calving elevated over the physiological level (0.2 mmol/l, Gönye, 1987, Gaál, 1999). The 0.183 mmol/l difference between the pre-calving NEFA concentration (Day -9) and that measured on Day 10 after calving proved statistically significant ($P<0.01$) in this group.

AST activity of the control and experimental cows ranged between 43.6-103.7 and 67.8-95.4 (U/l), respectively with no significant between group difference. The within group proportion of cows that had AST activity higher than 100 U/l was bigger in the control than in the experimental group (e.g. 55.5% vs. 33.3% on day 10, or 37.5% vs. 22.2% on day 107).

Prior to parturition the average urea concentration of the blood in the experimental cows was significantly higher than that of the control cows. After parturition the blood concentration of the urea in both groups have elevated slightly over the accepted physiological limit value (3.3-5 mmol/l, Brydl, 1993). The difference between the control and experimental cows proved statistically significant on day 22 after calving. Urea concentration of the urinal samples varied in both groups within the physiological range (130-300 mmol/l, Vrzgula, 1985, Brydl, 1993).

Laboratory findings concerning the acid-base metabolism of the experimental and control cows indicated the presence of balance throughout the experiment. The average Net Acid-Base Excretion (NABE) was higher in both groups than the physiological limit value (>100 mmol/l, Kutas, 1965), while the urinary pH values remained within the range of pH 7.8-9.2.

Milk production: The experimental cows produced more milk from the very beginning of the lactation. The difference between the groups varied between 0.49 and 3.43 l/day.cow in favour for the experimental groups with an overall surplus of 2.14 l/day.cow. Experimental cows produced

somewhat more (0.09%) butter-fat in the average of the experimental period. No difference was found with respect to the milk protein content.

Feed intake: The experimental cows ate more Total Mix Ration (TMR) in the first half of experiment (4 decades). The feed consumption of the experimental cows in this period proved significantly higher ($p<0.001$) than that of the controls. The TMR intake was numerically more in the 6th and 7th decades in the experimental cows, and in the 8th decade the difference between the experimental and control cows became statistically significant ($p<0.001$). At the end of the experiment (9th, 10th, 11th decades) the control cows consumed more TMR ($p<0,001$). At the end of the experiment the experimental cows consumed less TMR for production of 1 litre of milk.

Body condition of the cows: At the beginning of the experiment there was only 0.2 score difference between the groups in favour of the controls, which then became less and from about the third samplings the condition of the experimental cows increased over the controls, but the 0.2-0.4 score difference was statistically not significant. Body condition of the cows reached minimum at about 32 ± 4.6 days after calving and proved inferior throughout the experiment to the score taken prior to calving. The about 30% difference between the body condition scores of the dry (experimental and control) cows and those measured at about 32 days after calving proved statistically significant ($P<0.001$). The condition scores of the experimental cows never went below 2.6 (to the contrast of the controls), and the decline of body condition in the post-parturient period was about 20% less ($P<0.001$) in the experimental group.

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