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EFFECTS OF A NON-STARCH POLYSACCHARIDASE ENZYME PREPARATION FROM *THERMOMYCES LANUGINOSUS* ON ENERGY AND PROTEIN METABOLISM AND MILK YIELD OF DAIRY CATTLE

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Non-starch polysaccharides (NSPs) form an integral part of the cell walls in plants and represent considerable available energy when degraded into absorbable mono-, di-, tri- and oligosaccharides. The ruminal microflora hydrolyses a good part of NSPs, however, recently there have been attempts to enhance the rate of utilisation by using external polysaccharidase enzymes. In the present study the effects of an enzyme preparation (Rumino-Zyme) high in xylanase activity were studied on ruminal volatile fatty acid (VFA) concentration, parameters of energy and protein metabolism, milk yield, feed conversion ratio (FCR) and body condition score of high-yielding dairy cows. A lignolytic enzyme preparation produced by the thermophilic fungus *Thermomyces lanuginosus* was applied in the present experiment and fed to dairy cows at 34 g/day dosage in the period between calving and the 110th day of lactation. This preparation increased VFA concentration in the rumen from about 32 days after calving and onward. Increased VFA concentration was followed by an about 5 to 10% increase in milk production and an almost 0.1% increase in butterfat production. Increased VFA concentration produced more balanced energy metabolism in the experimental cows as indicated by the lower incidence rate of hyperketonaemia, and lower acetoacetic acid and non-esterified fatty acid (NEFA) concentration in the blood of the experimental cows. Aspartate aminotransferase (AST) activity was tendentiously higher in the control group and the proportion of 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 concentration contributed to an improvement in energy balance in the experimental cows with a resultant improvement of feed intake and feed utilisation. Due to the more balanced energy metabolism postparturient body condition loss of the treated cows was reduced.

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Key words: Dairy cattle, *Thermomyces lanuginosus,* Rumino-Zyme, volatile fatty acids (VFAs), acetoacetic acid, non-esterified fatty acid (NEFA), aspartate aminotransferase (AST), net acid-base excretion (NABE), milk production, body condition, feed conversion rate

Non-starch polysaccharides (NSPs) are an integral part of the plant cell wall and with regard to quantity these carbohydrates form major components in the epi- and pericarpium of grain seeds. The predominant NSPs are cellulose, arabinoxylans (pentosanes) and β-glucanates. Due to the lack of appropriate enzymes most NSPs are non-digestible for monogastrics and in some cases they may act as antinutritives.

Since the early 1960s there have been many attempts to compensate this physiological handicap of monogastric animals by using enzyme preparations of different (mostly microbial) origin to improve production through the degradation of NSPs and phytates into available saccharides.

Through the ruminal microflora, ruminants use NSPs with agreeable efficiency. However, the idea to improve ruminal NSP fermentation by external enzyme preparations came to the fore and the first promising experiments were reported from the beginning of the 1980s.

External enzymes are used in two ways. Firstly: enzyme pre-treatment of roughages and forages is considered very promising. Nakashima and Orskov (1989) reported improved digestibility, while others gave accounts of enhanced weight gain in beef cattle (Beauchemin et al., 1995) and improved dry matter intake, increased milk and milk protein production in dairy cattle (Stokes, 1992; Chamberlain and Robertson, 1992; Kung et al., 2000). Enzymatic treatment decreased the pH, xylose and sugar content of silage (Stokes, 1992). No data were reported, however, on whether these enzyme preparations also affected digestion in ruminants.

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 the feeding of ruminants, because, according to the prevalent view, these enzymes become inactivated in the rumen. 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 milk production by about 5 to 10% at the beginning of lactation (Kung et al., 1997; Nussio et al., 1997) and in other experiments in the middle of lactation (Lewis et al., 1999; Schingoethe et al., 1999). Schingoethe et al. (1999) reported an increment in the butterfat and milk protein production of the treated cows; however, they failed to explain the background of that 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 a positive energy balance such 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 ruminal volatile fatty acid production, fat and carbohydrate metabolism, energy balance and 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). Of the imperfect fungi, *Aspergillus oryzae*, *Aspergillus niger*, *Trichoderma viridis* and *Humicola insolens* have been known to produce fibrolytic enzymes (Chamberlain and Robertson, 1992; 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 *T. lanuginosus*. IUB ranking of the enzyme is: endo-1,4-β-xylanase, 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 2000 IU/g (IU: one unit of xylanase activity expressed as μmol of reducing (xylose equivalent) sugar released in one min). The preparation hydrolyses xylans and arabinoxylans into mono-, di-, tri- and oligosaccharides.

Place and time of the study

The experiment was carried out at a loose-housing dairy cattle farm of 2000 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 in the 2nd or 3rd lactation (with 8850 ± 215 l milk in the last lactation) were 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 the daily ration of the experimental cows contained 34 g enzyme preparation (Rumino-Zyme) in 1 kg Protavit Hepar superconcentrate from calving till the 110th day of lactation (for details see Tables 1 and 2). No enzyme preparation had been administered before calving. The total mix ration was changed twice during the experiment according to the technology used in the farm. The first change was with the parturition, while the second change took place 28 days after calving (Tables 1 and 2).

Table 1

Composition of the daily ration

Feed components (kg/day)	Dry cows		Early lactation		Peak lactation	
	control	experimental	control	experimental	control	experimental
Maize silage	14	14	14.5	14.5	15.0	15.0
Meadow hay	6.5	6.5	1.0	1.0	–	–
Alfalfa hay	–	–	3.0	3.0	3.0	3.0
Alfalfa haylage	–	–	2.5	2.5	4.5	4.5
Chopped alfalfa	–	–	2.5	2.5	4.5	4.5
Wet sugar beet slices	–	–	1.5	1.5	3	3
Beer brewery residue	–	–	1.0	1.0	1.5	1.5
Molasses	–	–	0.6	0.6	0.6	0.6
Concentrate	0.5	0.5	–	–	–	–
Lactofort concentrate	–	–	1.5	1.5	1.6	1.6
Dairy-1 meal	–	–	6.5	6.5	9.5	9.5
Propylene glycol	–	–	0.1	0.1	0.1	0.1
Protavit Hepar	1.0	1.0	1.0	1.0	1.0	1.0

Table 2

Nutrient content of the daily ration

Nutrients	Dry cows		Early lactation		Peak lactation	
	control	experimental	control	experimental	control	experimental
Dry matter (kg)	12.0	12.0	19.5	19.5	23.4	23.4
NE_l (MJ)	65.9	65.9	141.3	141.3	168.1	168.1
Crude protein (g)	1448	1448	3602	3602	4244	4244
Crude fat (g)	314.5	314.5	1202	1102	1293	1293
Crude fibre (g)	3071	3071	2992	2992	3509	3509
Ca (g)	74.9	74.9	165.6	165.6	209.7	209.7
P (g)	72.5	72.5	95.8	95.8	120.7	120.7
Mg (g)	35.8	35.8	69.8	69.8	93.7	93.7
Na (g)	35.4	35.4	39.6	39.6	51.1	51.1
Vitamin A (1000 IU)	157.5	157.5	149.4	149.4	170.6	170.6
Vitamin D_3 (1000 IU)	26.2	26.2	39.4	39.4	45.6	45.6
Vitamin E (mg)	1357	1357	468.8	468.8	506	506

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 at about two-week intervals from the beginning of the experiment (9 ± 4.6 days before the expected parturition) till its end (107 ± 4.6 days after parturition). The first sampling (before the calving) was carried out for checking the health status of the animals.

Samplings were always carried out 3–5 hours after the morning feeding. Ruminal fluid was obtained via Dirksen tube. Blood and urine samples (20 ml each) were taken from the subcutaneous abdominal vein and via metal catheter from the urinary bladder, respectively. Samples were cooled to 4 °C and transported to the laboratory for further analysis. Clotting of the blood was prevented with heparin.

VFA concentration of the ruminal fluid samples was measured by gas chromatography. Ruminal fluid samples were first centrifuged at 10,000 rpm for 10 min. Five ml of the supernatant was added to 1 ml 0.6 mol oxaloacetic acid. After 2 h of rest at room temperature the material was centrifuged again for 10 min at 10,000 rpm, then the quantity of VFAs was measured in a Perkin-Elmer Sigma 3B gas chromatograph. Length and diameter of the column was 2 m and 2 mm, respectively. The column was filled with Carbopack B-DA 80/120 4% CW20M. Temperature of the thermostat and the detector was 200 and 260 °C, respectively. Nitrogen gas was used as carrier at a flow rate of 60 ml/min.

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

Glucose concentration of the plasma 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) recommended by the International Federation of Clinical Chemistry (IFCC). These examinations were carried out by an Autohumalyser 900S Plus clinico-chemical analyser (Human GmbH, Germany). NEFA and acetoacetic acid concentrations of the blood samples were 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). Urinary pH was measured by a digital instrument (Radelkis OP-211/1, Radelkis Co., Budapest). NABE was estimated by the method of Kutas (1965).

Milk yield of the experimental and control cows was recorded by milkings and computed for daily production by a Pro Vantage™ 2050 Integrated Man-

agement System adopted to the Bou-Matic milking system. Butterfat and milk protein 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 Excel 98) for significance. The same test was used for analysing the data of ruminal fluid and blood or plasma samples.

Results

VFA concentration of the ruminal fluid

Acetic, propionic and n-butyric acid concentrations of the ruminal fluid samples are presented in Tables 3, 4 and 5.

Ruminal acetic acid concentrations of the groups (Table 3) 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, when the experimental cows produced significantly more acetic acid than the controls.

Table 3

Acetic acid concentration (mmol/l) of the ruminal fluid samples

Sampling (Day ± 4.6)	Control	Experimental	Difference (%)	P
1st: Day 9 prior to calving	50.70 ± 8.48	50.29 ± 7.43	0.81	NS
2nd: Day 10 after calving	62.58 ± 18.44	63.30 ± 8.97	1.15	NS
3rd: Day 22 after calving	69.51 ± 15.62	52.37 ± 9.72	24.65	NS
4th: Day 32 after calving	61.30 ± 18.51	59.99 ± 7.12	2.13	NS
5th: Day 58 after calving	65.69 ± 16.86	63.83 ± 7.87	2.83	NS
6th: Day 75 after calving	54.58 ± 9.17	71.14 ± 9.35	30.34	< 0.005
7th: Day 107 after calving	51.81 ± 6.28	75.84 ± 12.92	46.38	< 0.001

NS = not significant

The average propionic acid concentration of the experimental cows proved inferior to that of the controls at the 1st sampling, and in the first month of lactation there was no major difference between control and experimental cows (Table 4). From the 5th sampling onward, however, the propionic acid concentration showed a steady increase in the experimental animals over the controls.

Average acetate : propionate ratio in the ruminal fluid of the control cows was 2.7, 2.1, 2.4, 2.4, 2.9, 2.4 and 1.8 in the order of samplings. The relevant values of the experimental cows were as follows: 3.6, 2.3, 2.3, 2.3, 1.9, 2.1 and 1.4.

Table 4

Propionic acid concentration (mmol/l) of the ruminal fluid samples

Sampling (Day ± 4.6)	Control	Experimental	Difference (%)	P
1st: Day 9 prior to calving	19.10 ± 2.11	13.85 ± 2.76	27.46	< 0.001
2nd: Day 10 after calving	30.15 ± 9.61	27.91 ± 6.69	7.42	NS
3rd: Day 22 after calving	28.57 ± 6.20	23.27 ± 6.47	18.55	NS
4th: Day 32 after calving	25.49 ± 15.13	25.68 ± 10.02	0.73	NS
5th: Day 58 after calving	22.28 ± 5.02	33.23 ± 4.62	49.16	< 0.001
6th: Day 75 after calving	23.14 ± 3.32	34.63 ± 5.30	49.62	< 0.001
7th: Day 107 after calving	29.39 ± 5.57	53.91 ± 16.15	83.45	< 0.001

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 at the next two samplings this parameter of the experimental animals lagged behind that of the controls (Table 5). In the second part of the experiment (from the 4th sampling onward) the n-butyric acid concentration was higher in the ruminal fluid of the experimental cows in comparison with the controls.

Table 5

n-Butyric acid concentration (mmol/l) of the ruminal fluid samples

Sampling (Day ± 4.6)	Control	Experimental	Difference (%)	P
1st: Day 9 prior to calving	8.85 ± 1.54	8.64 ± 2.53	2.33	NS
2nd: Day 10 after calving	18.05 ± 5.15	4.79 ± 1.36	73.48	< 0.001
3rd: Day 22 after calving	13.90 ± 2.77	13.21 ± 4.22	4.93	NS
4th: Day 32 after calving	7.19 ± 4.11	11.84 ± 1.92	64.67	< 0.05
5th: Day 58 after calving	13.09 ± 4.38	13.94 ± 2.15	6.44	NS
6th: Day 75 after calving	11.06 ± 1.68	16.11 ± 2.67	45.77	< 0.001
7th: Day 107 after calving	12.66 ± 3.05	18.24 ± 4.49	44.1	< 0.01

The concentrations of the total VFAs (Table 6) followed the pattern of the three organic acids discussed above. There was a higher VFA concentration in the experimental cows in the second half of the experiment.

Table 6

Total VFA concentration (mmol/l) of the ruminal fluid samples

Sampling (Day ± 4.6)	Control	Experimental	Difference (%)	P
1st: Day 9 prior to calving	78.64 ± 9.99	72.78 ± 10.49	7.45	NS
2nd: Day 10 after calving	110.78 ± 29.42	96.00 ± 14.66	13.34	NS
3rd: Day 22 after calving	111.98 ± 20.51	88.86 ± 18.27	20.65	< 0.05
4th: Day 32 after calving	93.98 ± 33.17	97.51 ± 17.00	3.76	NS
5th: Day 58 after calving	101.06 ± 24.96	111.00 ± 13.59	9.83	NS
6th: Day 75 after calving	88.78 ± 11.40	121.88 ± 15.60	37.29	< 0.001
7th: Day 107 after calving	93.85 ± 14.55	147.99 ± 31.66	57.68	< 0.001

Energy and protein metabolism

Glucose concentrations of the blood samples of control and experimental cows ranged between 2.4–3.3 and 2.6–3.5 mmol/l, respectively, throughout the study with no statistically significant differences between control and treatment cows (data are not shown).

The blood acetoacetic acid concentrations of non-treated cows (Fig. 1) were higher than those of 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 an acetoacetic 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 days 10, 22, 32, and 58, respectively. The incidence of hyperketonaemia in the experimental group was considerably lower with 8.3 and 11.1% on days 10 and 22 after calving. There was not hyperketonaemia on days 32 and 58 in this group.

Figure 2 shows the NEFA concentrations of the plasma samples. The NEFA concentration in the plasma of the control cows on day 10 after calving was 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 differences between groups (data are not shown). 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).

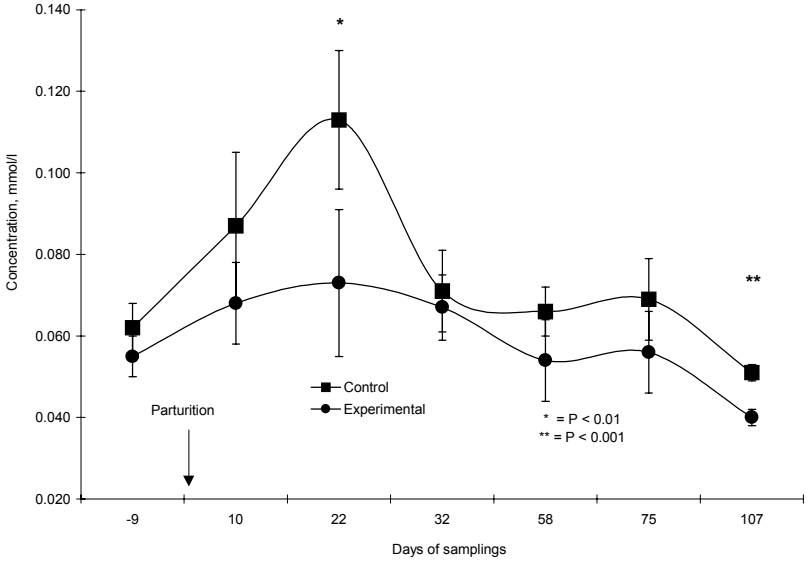

Fig. 1. Acetoacetic acid concentration of the blood samples

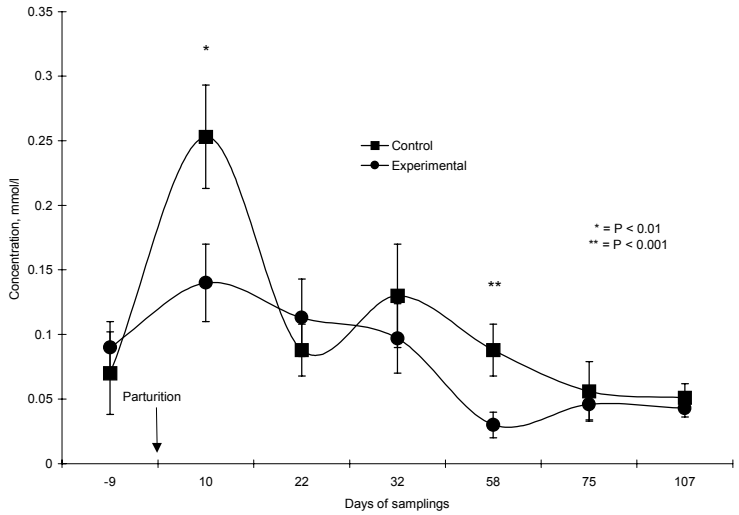

Fig. 2. Non-esterified fatty acid concentration of the plasma samples

Before parturition the average urea concentration of the blood in the experimental cows was significantly higher than that of the control cows (data are not shown). After parturition the urea concentration of the blood was slightly over the accepted physiological limit value (3.3–5 mmol/l, Brydl, 1993) in both groups. The difference between the control and experimental cows proved statis-

tically significant on day 22 after calving. Urea concentration of urine samples varied within the physiological range in both groups (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 (data are not shown) remained within the range of pH 7.8–9.2.

Milk production

Milk production of the experimental and control cows is summarised in Fig. 3 as daily averages of 10 day-periods (l/cow × day). Analysis of the data indicated within-group and individual variances, however, in the 1st, 5th, 6th, 7th, 8th, 9th and 11th decades the average milk production of the experimental group was significantly higher than that of the control. 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 of the experimental groups with an overall surplus of 2.14 l/day × cow. The experimental cows produced somewhat more (3.5% in control vs. 3.59% in exp.) butterfat in the average of the experimental period. No difference was found with respect to the milk protein content.

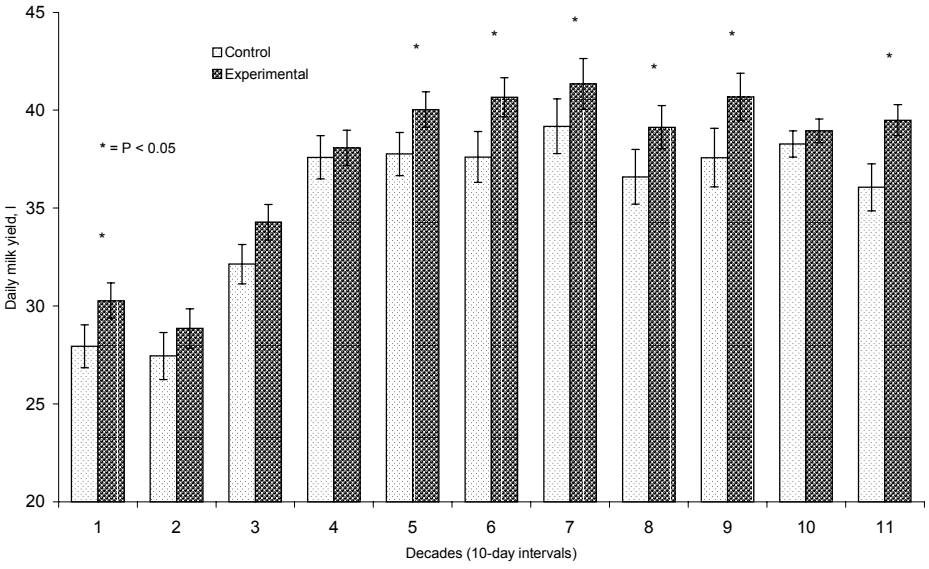

Fig. 3. Daily milk production in the average of 10-day production periods after parturition

Feed intake and conversion

The dry matter intake of experimental and control cows is shown in Fig. 4 as daily averages of 10-day periods (kg/cow × day). The experimental cows ate more feed in the first half of the 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 dry matter 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 DM ($P < 0.001$). The experimental cows consumed less DM for production of 1 litre of milk from the 5th decade till the end of the experiment (Fig. 5).

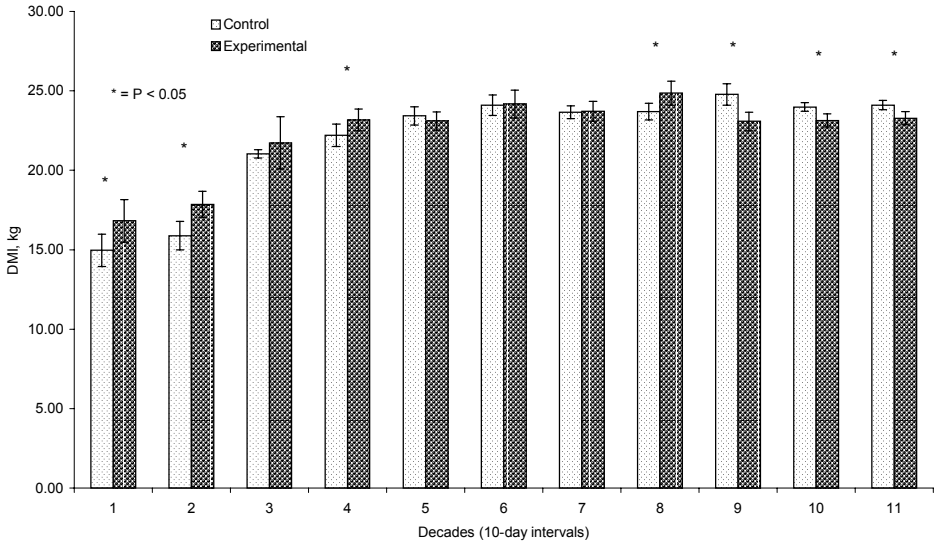

Fig. 4. Dry matter intake (DMI) in the average of 10-day periods after parturition

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 (Fig. 6), 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 mean condition scores of the experimental cows never went below 2.6 (in contrast to the controls) and the decline of body condition in the postparturient period was about 20% less ($P < 0.001$) in the experimental group.

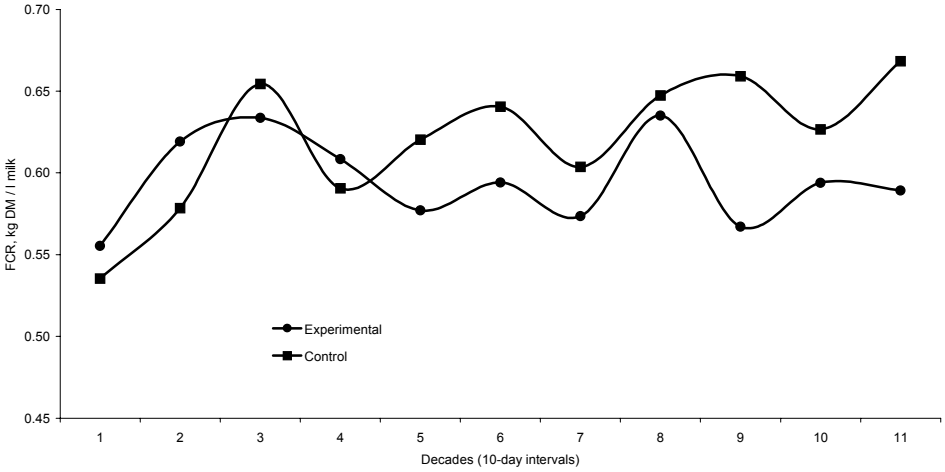

Fig. 5. Feed conversion rate (FCR) in the average of 10-day production periods after parturition

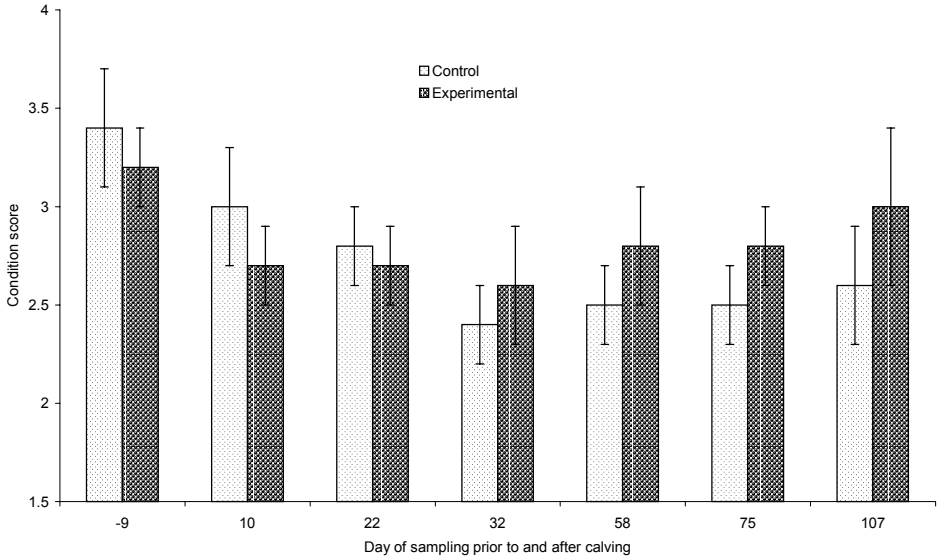

Fig. 6. Body condition score

Discussion

Negative energy balance prior to calving and in the early phase of lactation is common in high-yielding dairy cows with a consequent decline in milk yield and milk quality and with an increased rate of reproduction failures, clinical disease incidence and mortality. Subclinical ketosis and the subclinical fat mobilisation disease have their peak incidence in the first 3–4 weeks after calving (Brydl, 1999; Brydl et al., 2000).

In cattle, the majority of the energy requirement of tissues is covered by VFAs produced by the ruminal microflora from dietary carbohydrates. It is speculated that exogenous non-starch polysaccharidase enzymes act co-operatively with the endogenous enzymes (Morgavi et al., 2000) and this may enhance ruminal digestibility of fibre constituents resulting in more protein synthesis (Beauchemin et al., 2000) and in higher production of VFAs.

Few data are available on the effect of non-starch polysaccharidase enzymes on the ruminal VFA production. In the comparable experiment of Beauchemin et al. (2000) the quantity of ruminal VFAs did not change, but the proportion of acetate of the enzyme-treated cows increased over the control values. In the experiment using growing cattle (Krause et al., 1998) the concentration of VFAs in the ruminal fluid was of the same order in the enzyme-treated experimental and control animals, but the proportion of the propionic acid was higher in the treated cows. Contrary to the above findings Lewis et al. (1996) found an improved VFA production in beef cattle when the daily forage ration was supplemented with 1.65 ml/kg enzyme preparation of 5800 IU/ml xylanase activity.

In the present experiment the ruminal concentrations of VFAs were higher than the relevant control values from the time of the 4th sampling onwards. This finding indicates both the beneficial effect of the treatment on the ruminal VFA production and a considerable time of adaptation. The lower ruminal acetate:propionate ratio of the experimental cows found in the 2nd part of the present experiment seems to be in accordance with that reported by Yang et al. (1999).

Our results indicated more balanced energy metabolism and a reduced negative energy balance in the experimental cows in the first few weeks of lactation. This is proven, among others, by the higher blood acetoacetic and plasma NEFA concentrations of control cows right after parturition and by the increased incidence rate of hyperketonaemia in this group. AST activity indicative of liver cell damage was tendentiously higher in the control group and the proportion of cows that had AST activity higher than 100 U/l was also higher in the control group. The more intensive lipid mobilisation in the control group was proven also by the expressed decrease of the postparturient body condition score in the control group.

Milk production of the cows followed the pattern of the appetite of cows and the increased concentration of VFA in the rumen. The daily milk yield of the experimental cows was higher than the yield of the controls. From about 40–50 days after calving the milk production of the treated cows showed parallelism with the increased VFA production in the rumen. This experimental result supports those reported by others. Schingoethe et al. (1999) reported increased milk, butterfat and milk protein production as an effect of enzyme treatment and the response to the treatment was apparent after 2 to 4 weeks of treatment. In the experiment of Lewis et al. (1999) enzyme-treated cows (2.5 ml enzyme preparation/kg forage; xylanase activity: 5800 IU/ml) maintained higher milk production throughout the lactation following week 4 of the treatment. Yang et al. (1999) reported earlier response to enzyme treatment. In their experiment the diet of the experimental cows was supplemented with enzyme from the time of calving and the higher milk production of the treated cows was seen already in the 1st week of the experiment. Others (Sanchez et al., 1996; Nussio et al., 1997) also reported the positive effect of enzyme treatment on milk production. In the experiment of Luchini et al. (1997) the milk yield failed to increase, but the enzyme treatment caused higher milk-protein and butterfat production.

In contrast with the findings of Luchini et al. (1997), Rode et al. (1999) and Schingoethe et al. (1999) our data refer to a clear increase in the appetite of experimental cows. This finding of the present experiment supports the data published by Sanchez et al. (1996), Nussio et al. (1997), Lewis et al. (1999) and Beauchemin et al. (2000). Further to the increased daily DM intake our experimental cows consumed less DM for production of 1 litre of milk, indicating better FCR. Sanchez et al. (1996) and Luchini et al. (1997) also reported an improved FCR in enzyme-treated dairy cows. In the first phase of the experiment the daily milk yield shows parallelism with this better appetite of the experimental cows. The improved energy supplementation of the experimental cows resulted in more balanced body condition in the present experiment.

The mode of action of this preparation in the rumen is not clear in its details. This feed additive is a culture of the fungus *Thermomyces lanuginosus*, which possesses xylanolytic activity. It is speculated that the xylanolytic activity may be reduced a few hours after the administration, but the fungus survives and may have a *Saccharomyces cerevisiae* like effect in the rumen. This may explain the better appetite and the higher milk production after parturition in the experimental cows. A few weeks after the administration the fungus can multiply in the rumen and produce enough xylanase to improve fibre degradation. This may explain the better feed conversion ratio in the experimental cows at the end of the experiment.

Conclusions

Enhanced hydrolysis of NSPs in the rumen might be beneficial for the production of high-yielding dairy cows. The lignolytic enzyme preparation applied in the present experiment and fed to dairy cows at a dosage of 34 g/day increased the appetite of experimental cows and VFA concentration in the rumen from about 32 days after calving onward. Improved appetite and increased VFA production were followed by an about 5 to 10% increase in milk production and an almost 0.1% increase in butterfat production. The increased rate of VFA production contributes to the improved energy balance with a resultant improvement in feed intake and feed utilisation. Due to more balanced energy metabolism the postpartal weight loss of the treated cows is reduced, which decreases the risk of clinical manifestation of fat mobilisation syndrome.

Data of the present experiment supply further proof for the beneficial effects of lignolytic enzyme preparations in dairy cows. The inconsistent results of the studies quoted and discussed in this paper might be attributed to a number of factors including diet composition, type of enzyme preparation, amount of enzyme provided, method of application, stability of the enzyme etc. (Yang et al., 1999) and justifies further studies.

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