EFFECT OF PROCESSED STARCH-RICH GRAINS SUPPLEMENTATION ON RUMINAL FERMENTATION IN GRAZING, LACTATING DAIRY COWS

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SUMMARY

The effect of processed cereal grain supplementation on rumen fermentation pattern of grazing, lactating Holstein-Friesian cows was examined in a 5x5 Latin square experiment. The experimental treatments were the following: control (only grazing, no supplement addition), pelleted barley, pelleted maize, toasted and subsequently pelleted barley, and toasted and subsequently pelleted maize in 6 kg daily, equally two times proportioned. The rapid and extensive ruminal fermentation of starch in all supplemented grazing cows resulted higher concentrations of volatile fatty acids (VFA) and significantly lower ($P<0.05$) pH in ruminal fluid than in control animals. Total VFA (TVFA) concentrations (mmol) were higher for cows fed processed barley, than for cows fed processed maize. Supplement feeding significantly lowered ($P<0.05$) the acetate to propionate (C_2:C_3) ratio and the non-glucogenic to glucogenic ratio (NGR) in comparison to control animals, but the cereal grain type and processing method had no significant effect ($P>0.05$) on these ratios. The acetate molar proportion in supplemented animals decreased from 66% to 61% and the propionate increased from 19% to 24% regardless the type of the supplementation or the method of processing. Ruminal concentrations of ammonia (mg/l) and the ammonia to TVFA ratio significantly decreased ($P<0.05$) when the cows were supplemented with processed grains. Toasting and subsequently pelleting resulted lower ($P>0.05$) ruminal ammonia concentrations and lower ($P>0.05$) ammonia to TVFA ratio as well than did pelleting only. The different processing methods resulted in similar VFA patterns, C_2:C_3 ratios, and TVFA concentrations in the rumen. It is concluded that supplementing grass with heat treated cereal grains substantially elevates ruminal TVFA concentrations and reduces ammonia concentrations in the rumen.

ÖSSZEFOGLALÁS

Tóthi, R. – Taweel, H.Z.H. – Tamminga, S.: A KEMÉNYÍTŐBEN GAZDAG HŐKEZELT GABONAMAGVAK ETETÉSÉNEK HATÁSA A LEGELTETETT TEJELŐ TEHENEK BENDŐFERMENTÁCIÓJÁRA

A szerzők az előkészített gabonamagvak etetésének a legeltetett, Holstein-Fríz tejelő tehenek bendőfermentációjára kifejtett hatását 5x5-ös kezelésváltó latin négyzet kísérleti elrendezésben vizsgálták Hollandiában. A legeltetés során kizárólag legelőfüvet (Angol perje, *Lolium perenne*) fogyasztó kontroll kezelés mellett, különböző, hőkezelt gabonamagvakat etettek (pelletált árpa, tósztolt majd pelletált árpa, pelletált kukorica, tósztolt majd pelletált kukorica) napi 6 kg mennyiségben, két egyenlő részre osztva a reggeli és az esti fejések során. A gabonamagvak keményítőjének gyors és extenzív bendőbeli fermentációja minden abrakot fogyasztó tejelő tehén esetében a bendőfolyadék *nagyobb illózsírsav-koncentrációját és szignifikánsan alacsonyabb (P<0,05) pH értékét* eredményezte a kontroll állatokhoz képest. A bendőfolyadék összes illózsírsav koncentrációja (mmol/l) magasabb volt az árpával etetett, mint a kukoricával etetett állatok bendőjében. Az abraketetés következtében szignifikánsan csökkent ($P<0,05$) a bendőbeli ecetsav-propionsav (C_2:C_3) arány és a nem glükogén-glükogén illózsírsavak aránya, azonban a gabonamag típusa vagy a gabonamag előkészítésének módja nem volt szignifikáns hatással ($P>0,05$) a bendőfermentáció ezen paramétereire. A bendőemésztés során a gabonamag típusától vagy az előkészítés módjától függetlenül a kontroll állatokéhoz képest fokozódott ($P<0,05$) a propionsavas erjedés (a propionsav moláris aránya 19%-ról 24%-ra nőtt), ami az ecetsav csökkent mértékű ($P<0,05$) keletkezésével járt együtt (az ecetsav moláris aránya 66%-ról 61%-ra csökkent). A különböző gabona előkészítési eljárások azonban a bendőben azonos illózsírsavak képződését, azonos ecetsav-propionsav arányt és azonos összes illózsírsav-koncentrációt eredményeztek. Az ammóniakoncentráció (mg/l), vala-

mint az ammónia-összes illózsírsav arány az abrakot fogyasztó állatok bendőjében szignifikánsan csökkent (P<0,05). A csökkenés mértéke tósztolt gabonamagvakat fogyasztó teheneknél jelentősebb volt, mint a pelletált abrakkal etetett tehenek esetében. A kísérlet alapján megállapítható, hogy a legelőfű kiegészítése pelletált, illetve tósztolt és pelletált árpával vagy kukoricával, a bendő összes illózsírsav mennyiségét növeli, míg az ammónia koncentrációt csökkenti.

INTRODUCTION

High ammonia levels in the rumen of dairy cows are an indication for a shortage of energy availability or a lack of synchrony between energy and nitrogen supplies, that limits the use of available nitrogen by ruminal micro organisms (*Huntington,* 1990). Synchronising the ruminal fermentability of energy (starch) and nitrogen sources increases the outflow of bacterial protein from the rumen of dairy cows (*Huntington,* 1997). Depending on the efficiency of microbial growth, the ratio between microbial biomass and their end products may vary between 0.4 and 1.0 (*Hvelplund,* 1991). When degradation of carbohydrates and proteins (in g per unit of time) are synchronised and take place in a ratio of approximately 5:1, microbial protein synthesis will occur most efficiently and with little nitrogen losses from the rumen (*Tamminga et al.,* 1990). The rate of degradation largely determines the ratio in which volatile fatty acid (VFA) are formed, rapid degradation usually means a high proportion of propionic acid (sometimes lactic acid may accumulate), whereas slow degradation results in the formation of predominantly acetic acid. The ratio in which VFA are produced depends on the chemical composition and the rate of degradation of the substrate (*Murphy et al.,* 1982), further on the rumen pH. A low rumen pH or a rapidly degradable starch enhances propionate production, whereas a high rumen pH, slowly degradable substrate and fibre enhances the production of acetic and butyric acid. The rate at which VFA are produced in the rumen will to some extent determine their molar concentration in the rumen liquid, which in turn influences their rate of absorption (*Dijkstra et al.,* 1993). Rate of absorption of VFA appeared to depend on VFA concentration, rumen pH and rumen volume. The ratio in which propionic acid, acetic acid and butyric acid are provided does have a severe effect on milk fat content (*Sutton,* 1989). Grazing of very lush pasture will stimulate grass intake, but crude protein from this pasture is usually high, and rapidly and extensively degraded in the rumen (*Lopez et al.,* 1991). Furthermore high forage diets promote extensive absorption of ammonia from the rumen, because a greater proportion of ammonia is in the non-ionised form due to the higher pH associated with such diets (*Siddons et al.,* 1985). Under such conditions 50% of the crude protein ingested with the grass may be converted in the rumen into ammonia, absorbed in the blood stream, being converted into urea in the liver and excreted in the urine, resulting in poor utilisation of pasture protein. To reduce urinary N-losses and to improve the efficiency of milk N-synthesis in high yielding dairy cows, N-intake should be reduced without decreasing the energy intake (*Van Vuuren,* 1993). This can be achieved by the partial replacement of grass by low protein supplements high in non-structural carbohydrate, for instance cereal grains. Whole cereal grain with an intact pericarp is largely or entirely resistant to digestion by ruminants because whole kernels are resistant to bacterial attachment (*Beauchemin et al.,* 1994). Cereal

grains differ widely in their rate of degradation in the rumen and as such may not always match with the degradation of protein in pasture grass. Barley starch is degraded rapidly and almost completely in the rumen whereas often a substantial proportion of maize starch escapes rumen fermentation. A further optimisation is then possible by such a way of processing the grain, that the degradation of its starch becomes balanced to the degradation of protein in grass. Non-thermal processes (roller and hammermill) and thermal processes (*dry*: roasting, popping, micronizing and *wet*: autoclaving, steam-flaking, steam pelleting, expanding, extruding, toasting) can be used to manipulate the rate of starch (*Owens et al.*, 1986; *Theurer*, 1986) and protein degradation (*Cenkvári and Schmidt*, 1989; *Várhegyiné et al.*, 1991) and hence ruminal availability. The thermal treatments alters kernel structure, thus, enhancing the release of starch granules from the protein matrix and disrupting their order (i.e. of crystallinity) during gelatinization, resulting in increased susceptibility to enzyme activity (*Hoover and Vasathan*, 1994). Examination of the available literature clearly shows that the effects of wet-thermal treatments has a potential to manipulate the rumen degradability of the cereal starch in lactating dairy cows (*Tóthi*, 2002; *Tóthi et al.*, 2002). Thermal processing methods have been widely studied, with those of pressure toasting still being relatively recent.

The objectives of the experiment reported were therefore to investigate the effects of various ways of processing cereal grains as a supplement to grazing, high yielding dairy cows on patterns of rumen fermentation characterised by pH, ammonia and VFA concentrations in rumen liquid.

MATERIALS AND METHODS

Animals and management: The experiment was carried out at the experimental farm 'De Ossekampen' of Wageningen University, The Netherlands. Five multiparous lactating Holstein-Friesian dairy cows previously surgically fitted with a rumen cannula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) were used. Two cows were in their 7th and the others were in 2nd, 4th and 6th lactation, respectively. At the beginning of the experiment the cows produced 28.6±4.6 kg/day milk and averaged 173 days post partum. The animals were milked twice daily at 7:00 h and 17:00 h.

Experimental design: The experiment was a 5x5 Latin square design with five cows, five treatments and five periods in summer time. Each experimental period consisted of 14 days. Days 1–9 were used for adaptation and days 10–14 for sample collection.

Treatments and feed processing: The five experimental treatments were a control treatment of grass only (no supplement addition, NS), grass with pelleted barley (PB), grass with pelleted maize (PM), grass with toasted and subsequently pelleted barley (TPB), grass with toasted and subsequently pelleted maize (TPM). Grain processing was carried out at the Wageningen Feed Processing Centre (WFPC). A laboratory scale pressurised toaster was used for pressure toasting the grains for 1.5 minutes at 135 °C. After toasting, the grains

were dried in a forced air oven for 16 h at 35 °C, and followed by pelleting. Pelleting (80 °C, 10 s) was carried out with a 5x65 mm (bore x hole) die, using a V2-30 pelleting press (Robinson milling systems B.V., Boxtel, The Netherlands). The processed cereal grains (*Table 1*) were from the same bathes used in a previous experiment (*Tóthi et al.*, 2003a).

Table 1.

Chemical composition of processed grains and of perennial ryegrass

Grains(1)	Barley grain(2)		Maize grain(3)		Grass(4)
	PB	TPB	PM	TPM	
Dry matter, g/kg(5)	972.2	972.9	970.9	971.8	203.7
In dry matter, g/kg(5)					
Organic matter(6)	977.7	978.9	986.6	986.1	903.4
Crude protein(7)	114.9	113.7	92.0	92.9	211.2
Starch(8)	571.9	596.7	682.9	710.1	—
NDF	139.0	134.0	80.1	84.0	375.1
ADL	7.8	11.3	4.3	8.5	21.0

PB: pelleted barley(9), TPB: toasted and pelleted barley(10), PM: pelleted maize(11), TPM: toasted and pelleted maize(12)

1. táblázat: A hőkezelt abraktakarmányok és a legelőfű kémiai összetétele
gabonamag(1), árpa(2), kukorica(3), legelőfű(4), szárazanyag(5), szervesanyag(6), nyersfehérje(7), keményítő(8) pelletált árpa(9), tósztolt és pelletált árpa(10), pelletált kukorica(11), tósztolt és pelletált kukorica(12)

Experimental procedure and sample collection: In the 10-day-long adaptation period, the cows were allowed to graze freely with the herd in a pasture of perennial ryegrass (*Lolium perenne*). Next to a control treatment of grazing only, the four forms of processed grains were fed as a supplement in two equal portions of 3 kg each in the milking parlour during the morning (7:00 h) and evening milking (17:00 h). During the morning milking (7:00 h) of the 5-day-long experimental period, the cows consumed the heat treated grains in the milking parlour, then they were placed in their respective grazing plots (every morning new plots), tethered within a circular plot of a fixed area with a radius of 6 meters and allowed to graze in the morning from 8:00 h to 11:00 h. A detailed description of weather conditions during the experiment, sward management and sampling has been published elsewhere (*Tóthi et al.*, 2003a). Chemical composition of the available grass can be seen in *Table 1.* After 3 hours of grazing each cow was removed from the plot, brought to the barn and starved until 17:00 h, then milking and concentrate feeding was repeated. During the starvation period the animals had free access to water and mineral blocks (KNZ Liksteen). After milking, the cows were allowed to graze freely in experimental pasture of perennial ryegrass until next morning.

During the grazing time, starting at 8:00 h in the morning, samples of rumen fluid were taken every 30 minutes until 11:00 h and subsequently at 12:00 h, 14:00 h and 17:00 h (10 samples per cow per day). Ruminal fluid was obtained by suction from the ventral rumen compartment using a perforated rod and pH was immediately determined in the sample with a portable pH meter (pH electrode type 62, Testo 252, Testo GmbH & Co., Germany).

Chemical analysis and calculations: Processed cereal grains and grass samples were analysed for dry matter (DM), starch (except the grass samples), *nitrogen (N), neutral detergent fibre (NDF) and acid* detergent lignin (ADL) and ash. DM was determined by drying at 103 °C to a constant weight according to ISO-standard 6496, ash by combustion at 550 °C following ISO-standard 5984. Nitrogen was determined with the Kjeldahl method with $CuSO_4$ as the catalyst, according to ISO-standard 5983. ADL was analysed by the method of *Goering and Van Soest* (1970). NDF was determined by the VVR/protocol NSP analyses (*Anonymus*, 1992). This method is similar to the method of *Van Soest et al.* (1991), but includes an incubation step with 1 ml heat stable amylase (Sigma 6814, 1350 U/ml) and 0.25 ml protease (Alcalase, 2.4 L NOVO, 2.4 AU/g) in 60 ml phosphate buffer (pH 7). This incubation is carried out for 15 min at 40 °C after boiling and removal of the neutral detergent. Starch was analyzed according to the NIKO method (*Brunt*, 1992).

For ammonia analysis, 5 ml of the buffered rumen fluid were taken, to which 5 ml of 10% trichloroacitic acid were added. Ammonia concentration determined by transforming the ammonia with phenol and alkaline hypoclorite into indophenol-blue. The concentration of indophenol-blue was measured spectrophotometrically at a wavelength of 623 nm (Beckmann DU 64, Soft Pac module Quant II. Beckmann Instruments, Inc, USA).

For VFA analysis, 10 ml of the buffered rumen fluid were taken, to which 0.5 ml of phosphoric acid 85% (Merck 573) was added. Samples were centrifuged at 10.000 g for 10 min. After centrifugation, 0.5 ml of the supernatant was taken, to which 0.2 ml water and 0.3 ml internal standard (4 gm of 4-methyl-valeric acid per litre) (Merck Art. 806088) were added. Samples were analyzed for VFA concentrations by gas chromatography (GC type Fisons HRGC *MEGA2), using a sample changer A200S. The column was 6 feet (183) long* with an inside diameter of 2 mm and had a temperature of 190 °C; direct injection in the column with flame ionisation detector (FID). The temperature of the injector was 180 °C and of the detector 225 °C; the carrier gas flow was pure nitrogen saturated with formic acid. Data analysis software was chrom card –Thermoquest Italia.

The VFA's analysed were acetic acid (C_2), propionic acid (C_3), butyric acid (C_4), isobutyric acid (iC_4), isovaleric acid (iC_5), and valeric acid (C_5). The total concentration of VFA (TVFA) in the rumen fluid was calculated as the sum of C_2, C_3, C_4, iC_4, C_5 and iC_5. The non-glucogenic to glucogenic ratio (NGR) of VFA's was calculated as the ratio between C_2+ $2C_4$ and C_3 (*Ørskov*, 1975).

Statistical analysis: The *experimental data were analysed using the* PROC GLM procedure of *SAS* (1995). Cow, period and treatment (supplementation, grain, heat and the interaction between heat and grain) were the class variables in the model. When significant differences due to the treatment were detected, the multiple comparison procedure (Tukey) were used. Results are reported as least square means and standard errors of least square means. Treatment effect within feed type was judged using PDIFF in SAS 6.12 (*SAS*, 1995). Differences of $P<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Ruminal pH and VFA concentrations: The rapid and extensive ruminal fermentation of starch in all concentrate fed grazing cows resulted in higher concentrations of TVFA in ruminal fluid than in non-supplemented (NS) animals (*Table 2*). However significantly ($P<0.05$) only NS and toasted and subsequently pelleted barley (TPB) differ. The high concentrations of TVFA in ruminal fluid depressed the pH of ruminal liquid (*Fig. 1*) in comparison with NS animals to values that averaged around 6.1 during the experimental day for each supplemented animals and tended to be slightly lower for the barley than for the maize (*Table 2*). About 30 minutes after starting of the grazing period, ruminal pH of the supplemented animals continuously decreased in the grazing phase (*Fig. 1*) to below 6.0 for about 2 hours which may have affected the effective function of enzymes necessary for fibre breakdown and the cellulolytic activity of bacteria and depressed ruminal fiber degradation.

Table 2.

Rumen fluid pH and VFA concentrations (mmol) and molar percentages (mol/100 mol) in dairy cows grazing (9 h) grass pasture, and supplemented with concentrates

	NS	Barley grain(1)		Maize grain(2)		SEM	P		
		PB	TPB	PM	TPM		G	H	GxH
pH	6.35[S]	6.10	6.09	6.11	6.13	0.06	0.8	0.9	0.7
TVFA(3)	114.1[TPB]	121.0	125.3	121.7	115.5	3.3	0.2	0.8	0.14
Acetate (4)	66.3	60.9	61.3	61.2	60.6	2.7	0.3	0.8	0.2
Propionate(5)	18.7	23.6	22.9	23.9	23.9	1.1	0.8	0.5	0.4
Isobutyrate(6)	0.98[S]	0.71	0.73	0.73	0.74	0.04	0.6	0.8	0.3
Butyrate(7)	11.4	11.9	12.9	11.7	11.9	0.7	0.2	0.7	0.3
Isovalerate(8)	1.49	1.24	1.28	1.29	1.21	0.1	0.4	0.7	0.2
Valerate(9)	1.0[S]	1.5	1.42	1.23	1.51	0.2	0.4	0.6	0.5
$C_2:C_3$	3.6[S]	2.7	2.7	2.6	2.6	0.14	0.7	0.9	0.9
NGR	4.8[S]	3.7	3.8	3.6	3.7	0.2	0.7	0.8	0.9

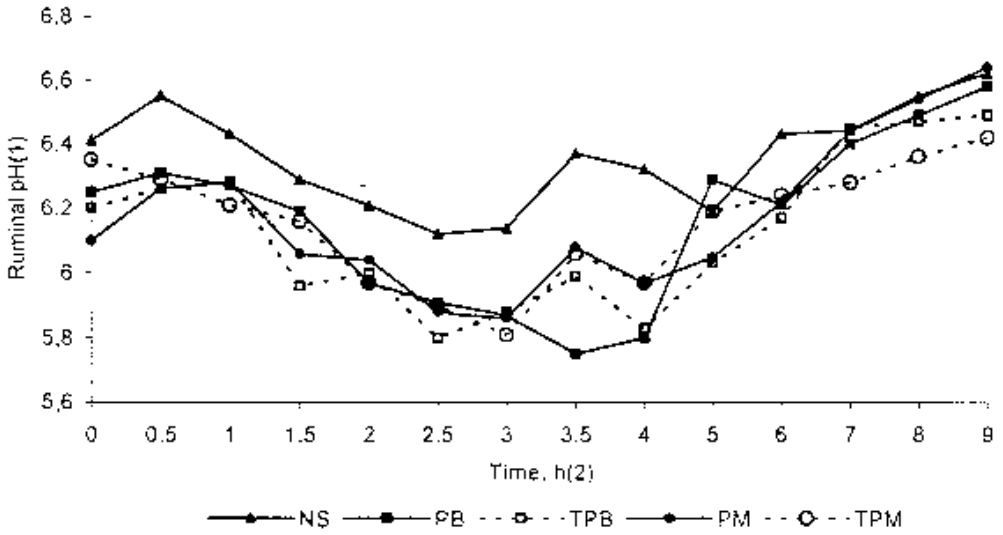
NS: no supplement addition(10), PB: pelleted barley (11), TPB: toasted and pelleted barley(12), PM: pelleted maize(13), TPM: toasted and pelleted maize (14), G: effect of grain type(15), H: effect of type of heat treatment(16), GxH: effect of grain type and heat interaction(17), $C_2:C_3$ ratio of acetate to propionate(18), NGR: non-glucogenic to glucogenic VFA ratio(19), [TPB] significantly ($P<0.05$) different from TPB treatment(20), [S] significantly ($P<0.05$) different from the other treatments(21)

2. táblázat: A legelő, illetve legelő és abrakot is fogyasztó tejelő tehenek bendőfolyadékának pH-értékeinek, az összes illózsírsav-koncentrációinak és az illózsírsavak moláris arányának alakulása a 9 órás legeltetés során

árpa(1), kukorica(2), összes illózsírsav(3), ecetsav(4), propionsav(5), izo-vajsav(6), vajsav(7), izo-valeriánsav(8), valeriánsav(9), nincs abrakkiegészítés(10) pelletált árpa(11), tósztolt és pelletált árpa(12), pelletált kukorica(13), tósztolt és pelletált kukorica(14), a gabonamag típusának hatása(15), a hőkezelés hatása(16), a gabonamag típusának és a hőkezelés kölcsönhatása(17), ecetsav-propionsav arány(18), nem glükogén:glükogén illózsírsav arány(19), [TPB] szignifikánsan különbözik ($P<0,05$) a TPB kezeléstől(20), [S] szignifikánsan különbözik ($P<0.05$) minden más kezeléstől(21)

Optimal pH for cellulolytic activity of bacteria in the rumen is near 6.8 (*Terry et al.*, 1969). Numerous *in vitro* studies have shown that the major cellulolytic bacteria (*Ruminococcus albus, Ruminococcus flavefaciens and Fibrobacter succinogenes*) cannot tolerate a pH below 6.0, because the bacteria are unable to maintain the pH inside their cells when ruminal pH is low (*Russell and Wilson*, 1996).

Fig.1.: **Changes of ruminal pH in grazing time (3 h) and the starvation time (6 h)**



for abbreviations see Table 1(3). NS: no supplement addition(4), each point represents the least squares mean of ten observations(5)

1. ábra: A bendőfolyadék pH-értékének változása a legeltetés (3 óra) és a koplalás (6 óra) időtartama alatt

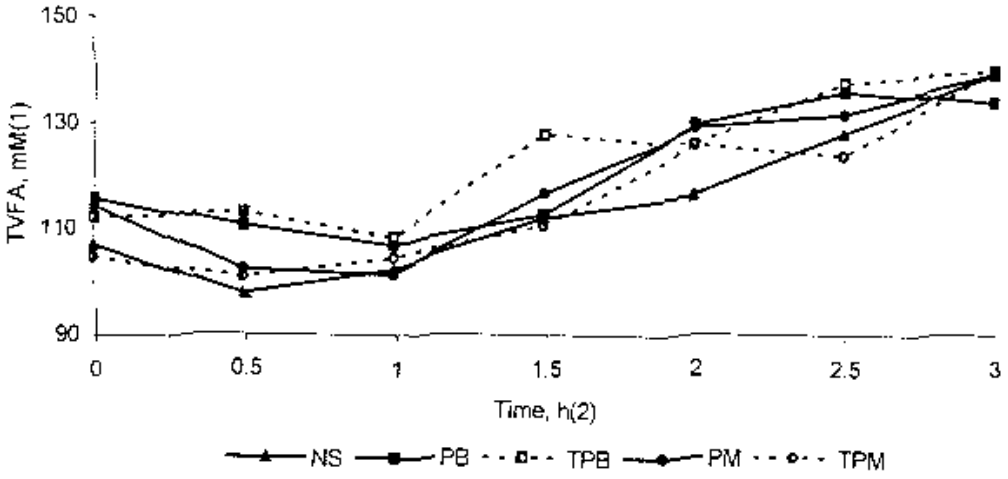
bendőfolyadék pH(1), idő, óra(2), a rövidítések az 1. táblázatban találhatók(3), nincs abrak-kiegészítés(4), minden pont tíz megfigyelésnek a legkisebb négyzetek módszerével számolt átlaga(5)

The effects of diurnal shifts in rumen pH *in vivo* are uncertain. *Yang et al.* (2000) measured ruminal pH and fibre digestion in dairy cows fed diets that ranged in the extent to which barley grain was flattened during steam-rolling and found that ruminal and total tract fibre digestion was unaffected by grain processing. Maybe other fibrolytic organisms contribute significantly to ruminal fibre digestion. *Forster et al.* (1999) found that over 60% of rumen bacterial species have not yet been fully characterised and some of these species may be fibrolytic.

The processing method did not have any significant effect ($P>0.05$) on the TVFA concentrations, especially when barley was fed. But when maize was fed, pelleted maize (PM) resulted in higher TVFA concentrations than when toasted and subsequently pelleted maize (TPM) was fed (*Fig. 2*). This was caused by the higher water soluble fraction of the maize starch after pelleting (39.6%) then toasting (35.0%) (*Table 3* dates based on the *in situ* experiments of *Tóthi et al.*, 2003b). Supplementation of pasture grass with heat treated cereal grains significantly increased ($P<0.05$) the concentration of propionic acid. There is a tendency of increased ($P=0.06$) butyric acid during grazing time, rising its daily ratio from 19% to 24% and 11% to 13% (*Table 2*). In the literature, major changes in the molar proportion of propionate (Sutton et al., 1987; *Visser et al.*, 1992; *Reis and Combs*, 2000; *Bargo et al.*, 2002) and butyrate (*Reis and Combs*, 2000; *Bargo et al.*, 2002) have been reported when rumen degradable starch was fed. The increase in propionate was mainly at the expense of the molar proportion of acetate decreased from 66% to 61% and to a lesser extent to isobutyrate of which the contribution decreased significantly from 1.1% to 0.9%.

Feeding supplements resulted in a significantly lower ($P<0.05$) acetate to propionate (C_2:C_3) ratio and glucogenic to non-glucogenic ratio (NGR).

Fig. 2.: **Changes of ruminal concentration (mmol) of total volatile fatty acid (TVFA) in grazing time (3 h)**



for abbreviations see Table 1(3), NS: no supplement addition(4), each point represents the least squares mean of ten observations(5)

2. ábra: A bendőfolyadék összes illózsírsav koncentrációjának változása (mmol) a legeltetés (3 óra) időtartama alatt
összes illózsírsav, mmol(1), lásd 1. ábra(2–5)

These decreases agree with those observed by *Van Vuuren et al.* (1986) for grass fed cows with 1 kg of a concentrate supplement vs. cows fed with 7 kg of a starch rich supplement. The increase in propionate due to supplying by grains the grazing cows is caused by the rapid degradation of starch by the amylolytic bacteria, which tends to produce propionate as its end product of utilising carbohydrates, because this produces more energy per unit of time. Amylolytic activity in the rumen is stimulated by a low pH while the acetate decreases due to the lower pH, which affects the activity and the efficiency of the cell wall degrading bacteria that produce mainly acetate. *Hoover and Stokes* (1991) suggested that the presence of an alternate, more readily digested carbohydrate could cause an initial inhibition of cellulose digestion. Also lower pH affects negatively the proteolytic activity of the rumen microbes and decreases protein degradation, this will cause a decrease in the branched chain VFA concentrations like isobutyric acid. *Stokes et al.* (1991), *Bach et al.* (1999) and *Ariza-Nieto et al.* (1998) found a higher molar proportion of propionate when increasing amounts of non structural carbohydrates were supplied to ruminal microbes that were maintained in a continuous culture system. *Dijkstra* (1994) concluded in his review that fermentation of structural carbohydrates compared to fermentation of starch yielded high amounts of acetate and low amounts of propionate.

Molar proportion of isobutyrate concentration was significantly higher ($P<0.05$) in NS animals than in supplemented animals and isovalerate showed also higher values. Since isobutyrate and isovalerate are the end products of protein degradation in the rumen (*Umbager*, 1978), these results indicate that rapid ruminal fermentation of starch in the rumen originating from heat treated cereal grains might have reduced protein losses.

The type of starch source and heat processing method had no significant effect ($P>0.05$) on the C_2:C_3 ratio, neither on the NGR ratio. The molar percent-

age of acetate concentrations were around 61% and the ones of propionate were around 24% regardless the type of the supplementation or the method of processing. These observations are in agreement with the data reported by *Casper et al.* (1999) and *DePeters and Taylor* (1985) who reported similar results when cows were fed diets based on either ground maize or barley. However *Casper and Schingoethe* (1989), *McCarthy et al.* (1989) and *Casper et al.* (1990) reported higher molar proportion of propionate for early lactation dairy cows that were fed barley compared to those that were fed with maize.

These similar molar percentages regardless the type of supplement, may be due to higher fractional passage rates of solids from the rumen for cows fed barley as reported by *Casper et al.* (1999), and due to the higher starch content of maize compared to barley (*Table 1*). *Joy et al.* (1997) investigated the concentration of VFA within the rumen for steam-flaked and dry-rolled maize, and found that the steam treatment increased the molar percentage of propionate whilst the concentrations of acetate and isovalerate declined. But as in this study the comparison is between two types of heat processing, no significant differences (P>0.05) were shown according to the processing method.

Ruminal ammonia concentration: Grazing pasture grass only resulted in significantly higher (P<0.001) ruminal ammonia concentration compared dairy cows supplying by grains all over the experimental day and in both phases of the experiment, grazing and starvation (*Table 4*).

Table 3.

***In situ* ruminal starch dissapearance of processed grains**
(Data based on *in situ* experiments of *Tóthi et al.*, 2003b)

	Barley grain(1)		Maize grain(2)	
	PB	TPB	PM	TPM
a (%)	57.0	49.5	39.6	35.0
b (%)	43.0^{a}	50.5^{a}	60.4^{c}	65.0^{c}
c (% h)	21.0^{a}	19.0^{a}	3.0^{b}	6.0^{b}
D (%)	88.6^{a}	86.7^{a}	65.0^{b}	65.7^{b}

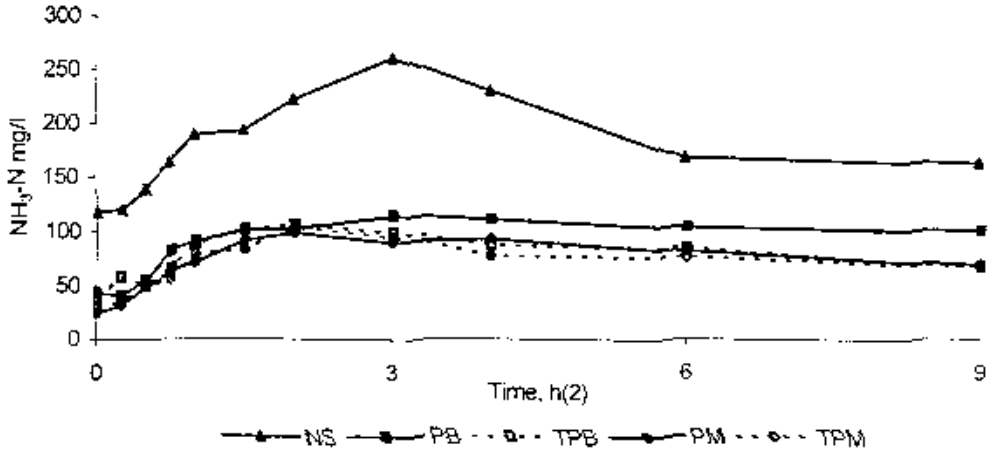
PB: pelleted barley(3), TPB: toasted and pelleted barley(4), PM: pelleted maize(5), TPM: toasted and pelleted maize(6), a: is water soluble starch(7), b: is non-soluble but potentially degradable starch in the rumen(8), c: is fractional rate of degradation (the part of b which is degraded per hour(9), D: is effective degradability, assuming a fractional rate of passage of 0.06 h(10), $^{a\,b\,c}$ figures with different superscript in the same row differ significantly (P<0.05)(11)

3. táblázat: A hőkezelt abraktakarmányok bendőbeli keményítőlebonthatósága (Tóthi és mtsai, 2003b in situ kísérletei alapján)
árpa(1), kukorica(2) pelletált árpa(3), tósztolt és pelletált árpa(4), pelletált kukorica(5), tósztolt és pelletált kukorica(6), a: vizben oldható, gyorsan lebomló keményítő hányad(7), b: lassan lebomló keményítő hányad(8), c: a lassan lebomló keményítőhányad bontási sebessége óránként(9) D: aktuális keményítőlebonthatóság, ha a bendőtartalom óránkénti kiáramlási sebessége 6%(10) az azonos sorban különböző betüvel jelölt értékek között szignifikáns különbség van (P<0,05)(11)

Higher values of ammonia concentration have been observed in grazing, non-supplemented lactating dairy cows (Van *Vuuren et al.*, 1986; *Rearte and Santini*, 1989). In agreement with the observations of *Chilibroste* (1999) the results of this experiments also show that in the morning grazing session after

the starvation during milking the ammonia concentration in the rumen liquid increased with time (*Fig. 3*).

Fig. 3.: **Changes of ruminal ammonia concentration (mg/l) determined in grazing time (3 h) and starvation time (6 h)**



for abbreviations see Table 1(3), NS: no supplement addition(4), each point represents the least squares mean of ten observations(5)

3. ábra: A bendőfolyadék ammónia koncentrációjának változása a legeltetés (3 óra) és a koplalás (6 óra) időtartama alatt
lásd 1. ábra(2–5)

The high ruminal ammonia concentration caused by pasture grass grazing supports the hypothesis that the protein of pasture grass is highly and extensively degraded in the rumen (*Beever and Siddons*, 1986; *Van Vuuren et al.*, 1986). The water soluble fraction of crude protein in perennial ryegrass is 55% and the potentially degradable fraction is 30% (*Tóthi et al.*, 2003b). *Siddons et al.* (1985) and Lopez *et al.* (1991) reported that protein from the pasture was rapidly and extensively degraded in the rumen, which resulted in high ruminal ammonia concentrations, of which a large proportion was in the non-ionised form because of the higher pH associated with feeding pasture grass. Unsupplemented cows had a peak in rumen NH_3-N at the end of grazing period (*Fig. 3*), indicating rumen proteolysis of pasture after a period of high grazing activity following the morning milking. In contrast, supplemented cows had a more constant pattern of NH_3-N in the rumen, indicating the improved utilization of NH_3-N by the energy provided with concentrate or a different diurnal pattern of grazing resulting from supplementation. Feeding heat treated grains decreased pH (*Fig. 1*) which also affects negatively the proteolytic activity of the microbes and decreases their ability to degrade protein. These results were in agreement with Hoover and Stokes (1991). Bach et al. (1999) found a decrease in ruminal ammonia concentration when they supplemented pasture with cracked maize and beet pulp. This decrease in ruminal ammonia was attributed to differences in bacterial N-utilisation and to the adequate amount of energy made available to the microbes to capture most of the ammonia from ruminal fluid.

The concentrations of ruminal ammonia with the supplemented diets were more than 50 mg/l reported by *Satter and Slyter* (1974) as the minimum ammonia concentration required in the rumen to ensure maximum microbial growth.

However *Russell et al.* (1983) found no differences in microbial growth when ammonia concentration in the rumen were below 50 mg/l or greater than 160 mg/l.

No significant differences (P>0.05) in ruminal ammonia concentration according to the starch source were found. But processed maize tended to have a greater effect than barley since it showed the lowest ruminal ammonia concentration all over the experimental day. However *Casper et al.* (1999) and McCarthy et al. (1989) found higher ruminal ammonia concentration when cows were fed maize compared with barley.

It was expected that barley would have a greater effect on the decrease of ruminal ammonia concentration than maize. Especially in the grazing phase due to the rapid degradation of its starch by microbes in comparison to maize as was reported by *Herrera-Saldana and Huber* (1990) and *McCarthy et al.* (1989). But as shown in *Table 4* maize had a greater effect than barley in both phases.

Table 4.

Ruminal ammonia concentration (mg/l) of dairy cows grazing grass pasture[1], and supplemented with processed cereal grains[2]

	NS	Barley grain(1)		Maize grain(2)		SEM	P		
		PB	TPB	PM	TPM		G	H	GxH
NH_3-N (D)[4]	180.9[S]	87.8	79.6	72.0	66.9	9.4	0.2	0.5	0.9
NH_3-N (G)	178.7[S]	81.7	78.4	67.3	66.2	8.8	0.2	0.8	0.9
NH_3-N (S)	185.7[S]	104.8	81.8	87.4	72.2	15.7	0.4	0.3	0.8
NH_3-N/TVFA ratio(3)	11.7[S]	5.4	4.6	4.5	4.5	0.5	0.1	0.5	0.8

[1] NS: no supplement addition(4); [2] PB: pelleted barley, TPB: toasted and pelleted barley; PM: pelleted maize, TPM: toasted and pelleted maize(5); G: effect of grain type(6); H: effect of type of heat treatment(7); GxH: effect of grain type and heat interaction(8); [4] D: all experimental day (9 h), G: grazing time (3 h), S: starvation (6 h)(9); [S] significantly (P<0.05) different from other treatments(10)

4. táblázat: A legelő, illetve legelő és abrakot is fogyasztó tejelő tehenek bendőfolyadékának ammónia koncentrációja (mg/l)

árpa(1), kukorica(2), ammónia-összes illózsírsav arány(3), nincs abrakkiegészítés(4), pelletált árpa, tósztolt és pelletált árpa, pelletált kukorica, tósztolt és pelletált kukorica(5), gabonamag hatása(6), hőkezelés hatása(7), a gabonamag és a hőkezelés interakciójának hatása(8), az egész kísérleti nap (9 óra), a legelés ideje (3 óra), a koplaltatás ideje (6 óra)(9), [S] szignifikánsan különbözik (P<0,05) minden más kezeléstől(10)

This is because barley has a higher proportion of ruminal degradable protein compared to maize which is the cause of higher ruminal ammonia when barley was fed. Also the higher concentration of starch in maize (*Table 1*) compared to barley caused the greater effect of maize on lowering ruminal ammonia concentration during the grazing phase. No significant differences (P>0.05) in the ruminal ammonia concentration were found between the processing methods. But in general toasting and subsequently pelleting had a greater effect in lowering the ruminal ammonia concentration than did pelleting only, regardless of the grains. This is due to the higher potentially degradable fraction caused by the pressure toasting and subsequently pelleting procedure compared to pelleting only (*Table 3*).

The ratio of ammonia to the TVFA was significantly higher ($P<0.05$) when the cows were not supplemented (*Table 4*). This was due to the higher ruminal ammonia concentration and the lower TVFA concentrations.

The high ammonia to TVFA ratio shows the unbalanced protein to energy characteristics of the feed. Supplementation lowered the ammonia to TVFA ratio from 12 to around 5. Because supplementing increased the TVFA production and concentration in the rumen and decreased the ruminal ammonia concentration all over the experimental day making feed more balanced and synchronising the availability of energy with rumen degradable protein. No significant differences ($P>0.05$) in ammonia to TVFA ratio were shown according to the heat treated grain source, neither to the processing method. Maize feeding had a slightly lower ratio (PM=4.5 and TPM=4.5) than barley (PB=5.4 and TPB=4.6). The lower ammonia to TVFA ratio caused by feeding processed maize was mainly due to the decrease in ruminal ammonia concentration. While in feeding processed barley, a moderate increase in the TVFA concentrations in addition to the decrease in ruminal ammonia concentration was the cause of the low ammonia to TVFA ratio.

CONCLUSIONS

The experiment demonstrated that the pasture grass as a sole feed for lactating, high producing dairy cows results high ruminal ammonia concentration and low ruminal VFA concentration. Pelleting as well as toasting followed by pelleting did affect rumen fermentation responses in lactating dairy cows by elevating TVFA concentrations and decreasing ammonia concentrations but no significant differences between the two heat treatments were found in this experiments.

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