THESISES OF DOCTORAL (PhD) DISSERTATION

UNIVERSITY OF WEST HUNGARY
FACULTY OF AGRICULTURAL AND FOOD SCIENCES
MOSONMAGYARÓVÁR
DEPARTMENT OF ANIMAL NUTRITION

Supervisor:
DR. SCHMIDT JÁNOS
corresponding member of the Hungarian Scientific Academy

IMPROVING GLUCOSE SUPPLY OF DAIRY COWS

Written by:
TÓTH TAMÁS

MOSONMAGYARÓVÁR
2005

1. INTRODUCTION

The high yielding dairy cow requires a great amount of glucose mainly to synthesize lactose, and for the synthesis of milk fat (to produce $NADP^+H^+$ via pentose phosphate pathway) and to maintain the nervous system as well. Dairy cows producing 30-50 kg milk per day require approximately 2.5-4.0 kg glucose daily (*Kutas*, 1987; *Reynolds et al.*, 1988; *Bergner* and *Hoffmann*, 1997; *Flachowsky* and *Lebzien*, 1997), but only a small amount (0.5-1.0 kg/day) of glucose is absorbed in the small intestine (*Flachowsky* and *Lebzien*, 1997). Plasma and liver glucose pool in a cow limited to 520-550 g, thus 1.0-3.0 kg glucose has to be synthesized by gluconeogenesis for the milk production mentioned. Thus, many feed additives (e.g. propylene glycol, Ca- and Na-propionate) or vitamins (e.g. niacin, biotin) are used in the practice to improve gluconeogenesis, and therefore increase the glucose supply of the high yielding dairy cow.

Besides improving gluconeogenesis, increasing the grain content of the diet is often used in practice. However, feeding high grain diets with high ruminally degradable starch content decrease rumen pH and dry matter intake, and modify the population of rumen micro-organisms. Moreover, these diets increase rumen propionate concentration, and therefore decrease the acetate to propionate ratio and fibre digestion (*Knowlton et al.*, 1996) and increase the incidence of rumen acidosis. A larger decrease in dry matter intake has been observed when rapidly fermentable starch sources, such as wheat and barley were fed to early lactation cows, and it was not the case when less degradable starch sources (corn or sorghum) were fed

(*McCarthy et. al.*, 1989; *Casper et al.*, 1990, *Moore et al.*, 1992; *Aldrich et al.*, 1993; *Chen et al.*, 1995; *Oliveira et al.*, 1995).

Chemical treatments of grains can be effective in avoiding the negative effects of the high starch diets, because they influence the ruminal degradation of starch, depending on the type and concentration of chemicals used.

As feeding concentrate has its own limits it is very difficult to set up a feed ratio that is capable to cover the energy requirement of high performance cows in the first period of lactation. As a result of insufficient gluconeogenesis the level of glucose synthesis limits milk production and can induce several metabolic diseases.

If the lactation performance of the cow increases the glucose production will limit milk yield more often. Therefore research studies were enhanced to improve glucose supply of cows.

2. OWN STUDIES

2.1. Objectives of the experiments

Utilising the long years experiences gained on improving bypass protein- and fat-products at the *Department of Animal Feeding* we aimed to elaborate process(es), which help to decrease ruminal degradation of starch in feedstuffs investigated in our experiments and to improve the glucose supply of the cows, respectively.

We chose the chemical treatments from the processes studied in the literature (physical, chemical, or combined physical+chemical) to reduce starch degradation in the rumen.

We wanted to answer the following questions in our experiments:

- What is the degradability of starch in cereal grains - grown in Hungary - in the rumen?
- Is there a significant difference in the degradation of starch in different corn hybrids in the rumen?
- Can NaOH- and NH_4OH- and different aldehydes (formaldehyde, glutaraldehyd, glyoxal) treatments be used to decrease ruminal degradation of the starch content in cereal grains (corn, wheat, barley, oat, rye, sorghum and triticale) being the most important feedstuff in the Hungarian feeding practice?
- What is the effect of the best chemical treatments on the main parameters of rumen fermentation?
- Which treatments and which doses are capable to pass the most post-ruminally digestible starch in the small intestine?
- Can milk yield increased by feeding sodium-hydroxide treated wheat?

- How does NaOH-treated wheat influence milk composition and the daily production of milk nutrients?

2.2. Materials and methods

2.2.1. Model studies with rumen, duodenal and ileocaecal cannulated steers

2.2.1.1. Starch degradability in the rumen using *in situ* method

The effect of different chemical treatments (NaOH, NH_4OH, glyoxal, glutaraldehyde, formaldehyde) on ruminal starch degradability of cereal grains (corn, sorghum, wheat, barley, rye, triticale, oat) was investigated using the *in situ* (in sacco) method. In the experiment, NaOH was used in the concentration of 2, 4, 6 and 8% and NH_4OH in the concentration of 1.5, 3 and 4%. In the case of glyoxal, glutaraldehyde and formaldehyde, the concentrations depended on the crude protein content of the grains. The crude protein content of corn and wheat were 90 g/kg and 120 g/kg, respectively (in % of feed), and the aldehydes were used in the concentration of 0.5, 1 and 2% of the grain's crude protein content.

Samples of ground grains weighing 100 g were treated with 25 ml of the different concentrations of chemicals and the chemically treated samples were dried at 60^{o}C in an exsiccator.

Later studies (*Flachowsky* and Lebzien, 1997) indicated that apart from cereal type there exist also differences in ruminal starch degradability between different corn varieties. In order to confirm this concept we examined 8 corn hybrids. They were as follows:

Very early maturity: *Dekalb 355*

Early maturity: *PR37M81*, *Asgrow 043*, *Dekalb 440*

Medium maturity: *Colomba*, *PR36R10*

Late maturity: *Florencia*, *Dekalb 557*

The experiment was performed using 3 rumen cannulated *Holstein Friesian* steers. *In sacco* bags were made from Scrynel plastic (*Zürcher Beuteltuchfabrick AG. Schweiz*) with a pore size of 40 micron. 2 g of ground grains were dosed per bag, thus the volume of material per 1 cm^2 of the surface of the bag was 13.9 mg. The incubation periods were 24 hours in all cases, and the measurements were done in 3 replications. In each replicate, 5-12 bags (which were determined in previous trials) were placed into the rumen through the fistula.

The incubation time was 0, 2, 4, 8, 16, 24 and 48 hours in the effective degradability trial. Each product was tested by animal and by incubation time in 5 replications. The effective degradability of the examined grains within the rumen was calculated with the following equation of *Kristensen et al*. (1982):

$$EDP = \sum_{i=0}^{n} [PD_{(ti+1)} - PD_{(ti)}] \times f_{(ti,\, ti+1)} + PD_o$$

where: PD = protein degradation

ti, ti + 1 = consecutive incubation times

$f_{(ti,\ ti\ +\ 1}$ = amount of protein in the rumen at the different incubation times

$f_{(ti)} = e^{-kp \times ti}$

$$f_{(ti,\ ti+1)} = 0{,}5 \times (e^{-kp \times ti} + e^{-kp \times ti+1})$$

$$i = 0, 2, 4, 8, 16, 24, 48 \text{ hours}$$

During the calculation the rumen passage (outflow) rate was 0.08/h (kr =8%) and obviously, the protein values were replaced with the adequate starch values.

Bags contained 2 grams of treated and untreated grains and were washed three times after the end of the incubation periods to clear away degraded nutrients and rumen fluid residues. After the determination of feed and starch remained in the bags, the ruminal dry matter and starch degradation of grains were calculated.

2.2.1.2. Effect on rumen fermentation and microbial protein

In this experiment, two rumen cannulated *Holstein* steers (650-660 kg body weight) were used in two replications and phased method to determine the effect of feeding wheat treated with NaOH or formaldehyde on rumen fermentation. The four-day treatment and control phases were conducted after 10 day adaptation periods. During the experiment, animals were fed 12 kg corn silage, 2 kg grass hay and 4 kg concentrate daily. During the NaOH and formaldehyde treatment phases, animals were fed 2 kg/day wheat treated with 2% NaOH- (as fed basis) or 2% formaldehyde (crude protein basis), therefore, the concentrate contained 50% treated and 37% untreated wheat.

During the control and treatment phases, rumen fluid samples were taken through the fistulae twice a day (before and 3 hours after the morning feeding). The pH, NH_3 and SCFA content (acetate, propionate, i- and n-butyrate, i- and n-valerate), and rumen microbial activity were analysed in the laboratory.

On the 5th days of the control and treatment phases, 3 hours after the morning feeding rumen fluid samples were taken to analyse the crude protein and DAPA (diaminopimelic acid) content of microbial protein.

2.2.1.3. Effect on postruminal starch degradation

The amount of starch reaching the small and large intestine was measured using two *Holstein* steers (500-520 kg body weight) fitted with rumen, duodenal, and ileocaecal cannulas in four replications. Feed rations and composition were same as in the rumen fermentation experiment. Similarly to the rumen fermentation experiment, this experiment consisted of four day collection periods following the 10 day adaptation periods. On the 1st and 4th days of the collection periods, chyme samples were taken in every two hours between 06.00 and 16.00 via the duodenal cannula and twice daily from the ileocaecal cannula.

Samples were collected using T-cannulas (not re-entrant), therefore TiO_2 was used as a marker to determine the flow of chyme in the duodenum and ileum. TiO_2 is a good qualified marker, not only because it passes through the intestine consistently and it is not absorbed in the intestine, but also its determination from the chyme is a quite simple method. A chyme

samples were analysed for pH, NH_3, DM, starch, crude protein, crude fibre, DAPA and TiO_2 content.

2.2.2. Laboratory analysis

The chemical content of the feeds (DM, crude protein, crude fibre, crude fat, crude ash, Ca and P) were analyzed according to the *Hungarian Feed Codex* (1990; chapter *5.1.*, *6.1.*, *7.1.*, *8.1.*, *10.1.*, *10.3.*, and *11.6.*; crude protein: *Kjeltec System 1026 Distilling Unit*, *Tecator Ltd.*; crude fibre: *Fibertec System M*, *Tecator Ltd.*; crude fat: *Soxtec System*, *Tecator Ltd.*; calcium and phosphorus: *Zeiss AAS 3*, *Carl Zeiss Jena*) Starch concentration of feed and chyme samples were analyzed with a polarimeter (*Carl Zeiss Jena*) as described in the *Hungarian Feed Codex* (1990; chapter *9.3.*). The pH and the NH_3 concentration of the rumen fluid were measured with electrical pH meter (OP-211/1, *Radelkis*) and ammonia sensitive electrode (OP-264/2, *Radelkis*), respectively.

Nitrite reduction probe with three different nitrite concentrations (0.2, 0.5, and 0.7 ml 0.025% KNO_2 solution/10 ml rumen fluid) was used to determine the microbial activity in the rumen fluid samples. Alfa-naftil-amine was the reagent (*Horváth*, 1979). Microbiological activity was calculated from the time, which was necessary for the reduction of nitrite by the rumen bacteria. The SCFA concentration of rumen fluid was measured using a gas chromatograph (Chrom-5, *Laboratorni Přistroje Praha*). Samples were centrifuged (15000/min), filtered, and treated with meta-phosphoric acid (25%) prior to injection. The columns were filled with

Supelco Carbopack™ B-DA (*Supelco Park*) wax. The duodenal and ileocaecal chyme samples were analyzed for TiO_2 using the method described by *Brandt* and *Allam* (1987). The TiO_2 content of the chyme was specified with spectrophotometer type Spekol (*Carl Zeiss Jena*) after destruction with sulphuric acid. 60 g of TiO_2 was fed as an indicating agent to the animals a day. In order to ensure the daily titan uptake is constant the TiO_2 was fed into the rumen directly via the cannula in 2 parts (2×30 g) a day. Knowing the daily TiO_2 dose and the TiO_2 content of chyme we calculated the amount of chyme passing through the duodenum with the following formula:

$$\textit{Chyme passing through the duodenum} \text{ (g/day)} = \frac{\textit{TiO}_2 \textit{ content of feed} \text{ (mg/day)}}{\textit{TiO}_2 \textit{ content of chyme} \text{ (mg/day)}}$$

To determine the amount of microbial protein synthesised in the rumen it is necessary to know the DAPA content of rumen microbial protein. Microbial protein was obtained from the rumen fluid using the method by *Krawielitzki* és *Piatkowski* (1977). According to this method we centrifuged the sample of rumen fluid and we inhibited the reproduction of microbes by formalin (20 ml/l rumen fluid) at 3000/min to remove the feed particles and protozoa from the rumen fluid. After that we isolate the rumen microbes from rumen fluid by centrifugalization at 16000/min. and we dried the microbial biomass by lyophilization.

To establish the amount of the microbial protein synthesised in the rumen we used DAPA as a marker. DAPA can be found in the cell wall of rumen microorganisms. We performed this examination using *Aminochrom-II* (*OE-914, Laboratóriumi Műszergyár Co.*) amino acid analyser and the

packing was *Kemochrom-9* (*Kemona Ltd.*) resin. We used the method described by *Csapó et al.* (1991) for this examination. In order to insure the good separation of DAPA we convert the metionine content of the sample into methionine-sulphone. We performed the oxidation with performic acid based on the method described by *Degussa* (Degussa Analitik/Analysis, 1986). Consequently, the DAPA appeared well measurably at the place of methionine between valine and isoleucine.

2.2.3. Field trial

A field trial (in Komárom at *Solum Co.)* was carried out to investigate the effect of feeding wheat grain treated with 3% NaOH (*Sodagrain*) to dairy cows on milk production and milk composition. 23-23 crossbreed *Holstein* cows (R_3, at the 2^{nd} period of their lactation) were involved in both the control and the experimental group. The milk production and other parameters were almost identical at the beginning of the experiment (*see table*).

Parameters in the field trial that were used for selecting the control and treatment cows

	Control	Experimental
	group	
Animal number in the control and experimental group	23	23
Average milk production in the previous lactation (L)	9141.2	9144.0
Lactation number (so far)	2.4	2.4
Days after parturition	100	101
Average daily milk production (L/cow)*	34.3	34.3

**During a two week period before the beginning of the trial*

The adaptation period took 2 weeks, and the experimental period was 4 weeks. During the experiment, cows were fed 2 kg/day treated (with 3% NaOH, whole grain) and untreated (grinding) wheat. The sodium-hydroxide treatment of wheat was carried out to use a *Keenan* (*Keenan Ltd.*) mixer-feeder. The treated wheat (*Sodagrain*) was fed to cows after 4 days. The buffer content of experimental ration was reduced by half-fold, because of the basic effect of the caustic soda-treated wheat.

The animals were milked twice a day at *Solum Co*. The milk samples were taken from both the morning and the evening milk on 2 days a week to specify the composition of the milk. The morning and the evening samples were individually combined in the proportion of the milked litre volumes before the testing.

The composition of the milk was tested by the *Magyar Tejgazdasági Kísérleti Intézet Ltd.* (Mosonmagyaróvár), during which the fat, protein, lactose, dry matter and solids-non fat contents of the milk were specified. The tests were made on a device type Milkoscan FT 120 (*Foss Electric*).

2.2.4. Statistical analysis

Data were analyzed using the t-test and correlation coefficient procedure of *STATISTICA 6.0.* and *MsOffice Excel* programs.

3. NEW SCIENTIFIC RESULTS

As a result of *in situ* and other metabolism studies with rumen, duodenal and ileocaecal cannulated steers and the data of dairy farm trials we can formulate the following new scientific achievements:

1. We were the first to examine ruminal starch degradability of different corn hybrids in Hungary. We concluded there is a significant difference in the rumen degradability of dry matter and starch of the corn hybrids of different maturity groups grown in Hungary.

2. There was a correlation of r=0,907 to be determined between the degradability of dry matter and starch in NaOH-treated corn, sorghum, wheat, barley, rye, oat and triticale, that equalled r=0,954 for corn hybrids. This level of close correlation and the degradation of the dry matter in the rumen led us to determine with high certainty degradability of starch in cereal grains and the rate of bypass starch (rumen degradable starch).

3. *In situ* experiments helped us to observe that treating corn and sorghum with 2, 4, 6 and 8% NaOH significantly increased the degradability of dry matter and starch in the rumen. At the same time if we treated wheat, rye, triticale, oat and barley with the different percentage of NaOH, a concentration of 2% significantly reduced the degradation of both the dry matter and the starch in the rumen.

4. As a result of *in situ* experiments NH_4OH applied at 1.5; 3.0 and 4.5% in corn, and wheat is not suitable to protect starch in the rumen. Although *in situ* treatments proved that glyoxal applied at 1 and 2% and/or glutaraldehyde and formaldehyde applied at 0.5; 1 and 2% depending on the crude protein content of corn and wheat significantly decreased the degradation of dry matter and starch in the rumen.

5. Feeding 2 kg/day wheat treated with 2% sodium-hydroxide (NaOH) or 2% (protein basis) formaldehyde did not affect negatively the main parameters of rumen fermentation (e.g. pH, NH_3, SCFA-production, microbial activity). Crude fiber degradation in the rumen was significantly improved when 2% NaOH-treated wheat was fed.

6. In experiments with rumen, duodenal and ileocaecal cannulated steers we proved that the feeding wheat treated with 2% NaOH and an amount of formaldehyde equivalent to 2% of the crude protein content significantly more starch passing to the small intestine and that surplus starch can be absorbed and as a result the glucose supply of the cows will improve.

7. In the feeding trial in a dairy farm whole grain wheat treated with 3% NaOH significantly increased milk yield, solids-non fat and protein content of the milk compared to the untreated ground wheat.

4. LIST OF THE PUBLICATIONS IN THE THEME OF THE PhD DISSERTATION

1. *Tóth, T.-Schmidt, J.* (2003): A glükóz ellátás jelentősége a nagy tejtermelésű tehenek takarmányozásában. Állattenyésztés és Takarmányozás, 52. 6. 557-571.

2. *Tóth, T.-Schmidt, J.* (2003): A fajta, valamint a nátrium-hidroxid kezelés hatása a kukorica és a gabonamagvak keményítőjének bendőbeli lebonthatóságára. Állattenyésztés és Takarmányozás, 52. 6. 573-581.

3. *Tóth, T.* (2003): A NAOH-dal végzett kezelés hatása a gabonamagvak keményítőjének bendőbeli lebonthatóságára. IX. Ifjúsági Tudományos Fórum, Keszthely, 2003. március 20.

4. *Tóth, T.-Schmidt, J.* (2004): Effect of different chemical treatments on ruminal starch degradability of corn and wheat. Acta Agronomica Óváriensis, 2. 177-185.

5. *Tóth, T.-Schmidt, J.* (2004): Kémiai kezelések hatása a kukorica és a búza keményítőjének bendőbeli lebomlására. XXX. Óvári Tudományos Napok, Mosonmagyaróvár, 2004. október. 7., 95.

6. *Tóth, T. – Beke, K. – Schmidt, J.* (2005): Nátrium-hidroxiddal kezelt búza etetésének hatása a tehenek tejtermelésére és a tej összetételére. Állattenyésztés és Takarmányozás (in press).