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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

PRODUCTION OF A PROTECTED (BYPASS) PROTEIN PRODUCT AND ITS USE IN THE FEEDING OF HIGH YIELDING DAIRY COWS

Written by:
SZOKOLY ZSUZSANNA

MOSONMAGYARÓVÁR
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1. Introduction

During the recent one and half decade the lactation production of cows has significantly increased in countries having developed cattle breeding. This development can be experienced both in Hungary and in Slovenia, since the lactation production of livestock involved in the production control increased from 4,875 litre to 7,618 litre between 1985 and 2003 in Hungary, and from 3,362 to 5,724 in Slovenia.

The growth of the milk production increased the nutrient need of the cows. First of all the energy and protein needs of the cows have increased. The increasing protein need of the cows cannot be covered exclusively via the increase of the protein content of the daily ration, since protein consumption beyond a certain limit impairs the fertile results. It has been proved by a number of experiments that there is a negative correlation between the crude protein content of the ration and the reproduction results (Kaufmann and Lüpping, 1978, Bruckental et al. 1996) This is explained by the fact that with the increase of the protein content of the ration – mainly if the daily ration containing lot of rumen degradable protein – the NH_3 content of the rumen fluid elevates which results in the increase of the urea content of the blood plasma, and consequently in the impairment of fertile results (e.g. sperm index, fertilisation %).

A solution is if the protein percentage in the daily ration is increased in proportion to the growth of the milk production which decomposes and assimilates not in the rumen but in the post-ruminally part of the digestive system.

This can be achieved by covering a part of the protein need with feedstuffs the protein in which degradability in the rumen to an extent less then the average 70%. With ration in which the degradability of the protein is 70% or above, the protein need of only 25-30 kg daily milk production can be covered without impairment of the fertile results. With the increase of the daily milk production the degradability of the daily ration has to be gradually reduced to 55-60% (Schmidt, 2003). According to Brydl (1998) it is advisable to provide 30-35% of the daily crude protein need in the lactation peak production period, 20% in the mid of the lactation, and 10% at the end of the lactation in the form of rumen protected (bypass) protein.

The fact that there is a limited number of feedstuffs among the Hungarian feeds of vegetable origin which have a high protein content and at the same time the degradability of the protein in the rumen is low launched the development of procedures (technologies) by means of which the degradability of the feed proteins in the rumen can be decreased in a way which does not impair or slightly impairs the postruminal digestion of the protein. Another important requirement to be met by the protected (bypass) proteins that they must have a favourable amino acid composition, since cows with high milk production receive relatively less microbial protein, however, high milk production requires perfect supply of essential amino acids in addition to other nutrients as well (Schmidt, 2003).

Considering that feeding of the ruminants with feedstuffs of animal origin is prohibited by veterinary rules aiming at the prevention of the spread of BSE currently the aim of the researches is to develop

protected protein products with bypass effect exclusively with the use of proteins of vegetable origin with valuable amino acid composition.

The above treatments can be classified into two groups: they may be physical or chemical procedures. Within physical treatments the heat treatment (e.g. extrusion, flaking), while within chemicals mainly the aldehydes (formaldehyde, glyoxal, glutaraldehyde) and the tannin are used for this purpose.

The lactation production of cows in milk is expected to continue to increase in the future, and consequently protein feeds will have an increasing role the protein of which degrades in the rumen to an extent essentially less than the average. This is due to the fact that the protein demand of the continuously increasing production may be met only with the increased use of such feedstuffs without the impairment of the reproduction results.

2. Aims of the studies

Based on the fact that the significant growth of the lactation production of the cows lead to the increase of the demand on protein feeds the protein of which degradability in the rumen to an extent less than the average, however, at the same time only a limited number of feedstuffs has been available in Hungary for the feeding of cattle since the prohibition of feeds of animal origin which meet this term, the aim of our experimental work is to develop a treatment procedure with which the degradability of the protein of the extracted soybean meal having a valuable amino acid composition may be essentially reduced without impairment of the postruminal digestibility of the protein.

We wished to find the followings during the experimental work:

- What is the influence of the treatment of the extracted soybean meal with hydrochloric acid on the degradability of the protein in the rumen?
- Can the influence of the treatment with hydrochloric acid decreasing the degradability of the protein be increased with heat treatment?
- Does the combined treatment (treatment with hydrochloric acid + heat treatment) influence the postruminal digestibility of the soy protein?
- Is the traditional heat treatment replaceable with heat-effect generated during the extraction of the product?
- What is the effect of feeding with the product made with the combined treatment on the microbial fermentation in the rumen?

- Does feeding with the extracted product of soybean meal made with the combined treatment influence the microbial protein synthesis?
- With what results can the procedure developed for the extracted soybean meal be used for the reduction of the degradability of the protein in the rumen in case of other feedstuffs rich in protein?
- What is the effect of feeding with the obtained product of soybean meal on the acid-base balance of the animals?
- How do the milk production of cows and the composition of the milk change in case of feeding with the extracted soybean meal made with the combined procedure?

3. Materials and methods

3.1. Treatment of the experimental feeds

During the experiments we wished to reduce first of all the degradability of the protein of the extracted soybean meal having a valuable amino acid composition in the rumen. We wished to achieve this with two methods, namely via treatment made with hydrochloric acid and combination of acid treatment and heat treatment.

The treated extracted soybean meal necessary for the material consumption experiments were made under laboratory conditions. During this the hydrochloric acid the volume and concentration of which was studied was sprayed onto the feed with several mixings of the soybean meal. As a result of the treatment the moisture content of the feed exceeds water content characteristic of the air-dry condition (23-25%), therefore desiccation is necessary. Desiccation was made

with two methods: by drying at 60° till an air-dry condition or by drying at 100°C for 30 minutes. In this latter case desiccation was a heat treatment supplementing the acid treatment at the same time. Treatment with hydrochloric acid as well as the subsequent heat treatment are hereinafter referred to as a combined treatment. Considering that the heat treatment further improved the efficiency of the treatment made with the hydrochloric acid experiments, during which the feeding value of the soy product made with the developed procedure was studied, were completed already with the product made with the combine treatment.

A greater volume of product that cannot be obtained under laboratory circumstances was necessary for the completion of experiments aiming at the establishment of the feeding value of the developed product (study of the effect of the product on the fermentation within the rumen, on the microbial protein synthesis, on milk production and on the composition of the milk), therefore a procedure was developed by us for the large-scale manufacture of the product. The task to be solved was the conduction of the heat treatment on a large scale. For this two solutions were studied by us. In the first case the heat treatment was made in the conditioning container of the Bocchi device with hot steam, at 100°C for 30 minutes. Then the product was desiccated and cooled in the drying unit of the Bochi device. The treatment of the extracted soybean meal with hydrochloric acid was made manually again. Already soybean meal treated with acid was transferred into the conditioning container

of the device. The experiment was made in the Mixing Plant of the Experimental Farm of Herceghalom.

Extraction of the soybean meal treated with acid was the other heat treatment procedure. The available extruder (device of Monex type) available for us was not suitable for the extraction of the extracted soybean meal in itself, therefore maize grain was added to the soybean meal treated with acid and to be extracted in a proportion of 30%. We could extract the mixture of 70% extracted soybean meal treated with acid+30% maize grain with excellent results. The temperature increased to 150°C after the extrusion. The extracted product was air-dry, and did not require additional drying. The volume of product necessary for the experiments was made in the extracting plant of the Galdorf Ltd. in Hernád.

3.2. Methods with animal tests

3.2.1. In situ method

The effect of the treatment made with the hydrochloric acid and with the combined method on the degradability of the protein in the rumen was tested with the *in situ* method. The experiments were made on 3 Holstein-Fries steers with cannula in the rumen and duodenum.

Bags used during the *in situ* tests were made from Scrynel plastic textile (Zürcher Beuteltuchfabrik AG. Schweiz) the porosity of which was 40 micron. We always added 2.0 g feed to be tested into the bags size 120×60 mm, thus the volume of material per 1 cm^2 of the bag surface was 13.9 mg.

In cases when the effect of the treatments on the degradability of the protein in the rumen was studied the incubation time was 24 hours. When we wanted to establish the effective degradability of the protein of the obtained product we incubated the samples in the rumen for 0, 2, 4, 8, 16, 24 and 48 hours.

We added the samples to be tested into 5 bags per treatment during each experiment. The experiments were repeated once during study of the effect of the treatment and twice during establishment of the effective protein degradability.

Bags containing the tested feedstuffs were tied to a 600 g iron ballast in order to dip them into the rumen fluid phase.

After the incubation the bags were washed with a shaker for 8×10 minutes, and the wash water was replaced with a fresh one on each occasion. After the wash the bags were dried in a drying box at 60°C.

The effective protein degradability of the tested products 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

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

$f_{(ti)} = e^{-kpxti}$

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

i = 0, 2, 4 8, 16, 24, 48 hours

During the calculation the rumen passage (outflow) rate was 0.08/h (kr =8%).

3.2.2. Establishment of the postruminal digestibility of the protein with a mobile bag technique

The postruminal digestibility of the protein was tested with the mobile bag technique. The material of bags used for this purpose was Scrynel plastic textile again, its size was 60×30 mm. 20 bags per treatment were tested. 0.5 g material was added into the bags than they were put into bigger bags used for the testing of the degradability in the rumen (3 mobile bags/rumen bag). After the subsequent 24-hour incubation the bags were washed, then 6 bags were cut up in order to establish the protein degradability in the rumen, while the remaining bags were subjected to a 48-hour in vitro digestion with hydrochloric acid - pepsin, then were placed via the cannula into the duodenum. The volume of the undigestible protein was established with the study of the feed remaining in the bags collected from the faeces.

3.2.3. Study of the effect of the product made with the combined treatment on the fermentation in the rumen

The tests were conducted with a periodic experimental method on 4 Holstein-Fries steers with cannula in the rumen. We wished to establish the effect of the product treated with acid and subjected to a traditional heat treatment in the 1st experiment then that of the product treated with acid and subjected to heat treatment with extrusion in the 2nd experiment on the fermentation in rumen.

The experimental phases (control and experimental phases) lasted for 5 days, while the preceding pre-feeding phases lasted for 9 days. Within the experimental phases we took samples from the rumen fluid via the cannula two times a day – before the morning feeding then 3 hours after the morning feeding, and we established its pH-value, NH_3 and volatile fatty acid content, and then studied the microbial activity of the rumen fluid sample.

3.2.4. Establishment of effect of the product made with the combined treatment on the microbial protein synthesis

The effect of the product made with treatment with hydrochloric acid then with heat treatment conducted via the subsequent extraction on the microbial protein synthesis in the rumen was studied on 2 steers with cannula in the rumen and duodenum within a periodic experiment. Within this experiment the control and the experimental phases lasted for 4 days, while a 10-day pre-feeding phase was applied before the testing phases. We took samples from the duodenal chymus via the duodenum cannula each two hours between 6 a.m. and 4 p.m. on day 2 and 4 of the experimental phase – on 6 occasions. On day 3 of the experimental phase we took a greater volume of sample - ca. 3 litre - from the rumen fluid via the rumen cannula. Microbial mass necessary for the establishment of the microbial protein synthesising in the rumen was obtained from it. The reason is that we must know its protein and DAPA content to be able to establish the volume of the microbial protein synthesising in the rumen.

In the experimental phase the extracted soybean meal and a part of the maize were replaced with a product obtained via extraction. The extracted product was 2.14 kg within the daily fodder dose. Within this the volume of the extracted soybean meal treated with hydrochloric acid was 1.5 kg.

Considering that the animals had a T-cannula instead of a re-entrant one, the use of an indicator was necessary for the establishment of the chymus volume passing through the duodenum. We chose titan-dioxide (TiO_2) as an indicator since in addition to the fact the it has all the necessary properties of an indicator (it smoothly passes through the digestive canal, does not assimilate, does not affect the digestion and the assimilation of the nutrients) it is easy to establish its volume in the chymus. 30 g of TiO_2 was added to a part of the feed (100 g feed) at each feeding (twice a day) and was forwarded directly into the rumen via the rumen cannula before the feeding. With this method our aim was to provide the necessary TiO_2 volume for the animals.

During the studies we established the pH-value, dry matter, raw protein, NH_3, DAPA as well as TiO_2 contents of the duodenal chymus samples.

3.2.5. The effect of feeding with the product obtained via the combined treatment on the acid-basis balance

Considering that 0.021 g HCl is forwarded to the rumen on feeding with the developed product via each g of the extracted soybean meal, we also studied in the framework of an experiment

what was the effect of feeding with the product on the acid-basis balance of the animals. For this purpose we took urine samples from the animals on each day of the phase in the 2nd experiment out of experiments described in subsection 3.2.2.3 - during which the effect of the product obtained with the combined treatment on the fermentation in the rumen was studied - both in the control and the experimental phases, and on the basis of the samples we established the net acid-basis emptied with the urine.

3.2.6. Large-scale milk production experiment

We set up a large-scale milk production experiment in the dairy of the Darnózseli Mezőgazdasági Co. to establish what is the effect of the extracted product of soybean meal made with the combined treatment on the milk production of the cows, on the composition of the milk and on the volume of the nutrients produced with the milk. The experiment was made with the so-called cow pair method. The following aspects were taken into consideration during pairing the cows:

- milk production in the first lactation,
- number of the finished lactations,
- lactation state (number of days from parturition)
- milk production at the beginning of the experiment

Based on the above aspects 28 pairs were made and one from each couple was included in the experimental group, the other in the control group. Parameters taken into consideration during the

selection of the pairs changed to parameters given in the following table within the two groups.

Parameters taken into consideration during the selection of the cow pairs in the large-scale experiment

Parameter	Control group	Experimental group
Number of cows in the group	28	28
Average milk production in the previous lactation, l	9779.58	9698.95
Number of lactations till now	2.65	2.54
Number of days from parturition	67.04	64.33
Daily average milk production at the beginning of the experiment, l/cow	33.66	34.26

The animals were accustomed to the ration fed in the experiment in a 8-day pre-feeding phase. The pre-feeding section shorter than the regular one (10-12 days) was enabled by the fact that the ration of the cows only slightly changed in comparison to the previously fed daily ration. After the pre-feeding phase the animals within the experimental groups ate 1.5 kg extracted product per day via 17 days - including 1.05 kg extracted soybean meal treated with hydrochloric acid - (experimental phase 1), then 2.0 kg extracted product per day via 16 days – including 1.4 kg extracted soybean meal treated with acid – (experimental phase 2) as part of the daily fodder dose. A part of the soybean meal and maize included in the ration also of the

control group was replaced with the product made with the combined treatment.

The animals are milked twice a day in the dairy. A milking plant connected to a computer operates in the plant and thus we had the chance to monitor the milk production of the cows by day and by milking.

The composition of the milk was tested once in a week by cow. The samples to be tested were composed from the morning and evening milking in the proportion of literage. During the test the dry matter, fat, protein, lactose and fat-free dry matter contents of the milk were established. The tests were made by the Magyar Tejgazdasági Kísérleti Intézet Ltd. (Mosonmagyaróvár) with a device type Milkoscan FT 120 (Foss Electric).

3.3. Chemical testing procedures used during the experiments.

The dry matter, crude protein, crude fat, crude fibre, crude ash, Ca- and P- contents of feedstuffs fed during the experiments as well as the dry matter and crude protein contents of the rumen fluid and of the duodenal chymus were established with the test procedures recommended in Volume 2 of the Hungarian Feed Codex (Chapters 5.1, 6.1, 7.1, 8.1, 10.1, 10.3, 11.6).

The pH-values of the rumen fluid and of the duodenal chymus were specified with an electronic pH-meter type OP211/1 (Radelkis) and their NH_3 contents with an ammonia-sensitive electrode type OP-264/2 (Radelkis).

The microbial activity of the rumen fluid was tested with a nitrite reduction probe, in case of 3 different nitrite concentrations (0.2, 0.5 and 0.7 ml/10 ml rumen fluid from 0.025% KNO_2 solution) (*Horváth,* 1979). Alpha-naphthyl-amine was used as a reagent. We made conclusions regarding the activity of the rumen microbes from the period which was needed by the rumen bacteria for their nitrite reduction.

The short-chain fatty acid content of the rumen fluid was specified with a gas-chromatograph equipment type Chrom-5. The rumen fluid was purified for the test first by centrifuging it at 15,000 rpm and by filtering it, then it was treated with oxalic acid of 0.3 mol before the injection - in accordance with the properties of the column fill. Supelco Carbopack^{TM} B-DA resin was the column fill. The water standard solution used for the identification contained the tested fatty acids with a concentration of 0.1 %.

The TiO_2 content of the chymus samples was specified with photometer type Spekol after destruction with sulphuric acid, with the method of *Brandt and Allan* (1987). Compound generating from the TiO_2 gives a yellow colour reaction in a sulphuric acid-phosphoric acid agent, with H_2O_2. The light absorption of the samples was measured at a wave length of 405 nm.

The volume of the chymus passing through the duodenum was established with the following equation with the known TiO_2 contents of the fodder dose and of the chymus samples:

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

The microbial protein was obtained from the rumen fluid with the method of *Krawielitczki and Piatkowski* (1977). The multiplication of the rumen microbes was stopped with formaldehyde (20 ml formaldehyde/1000 ml rumen fluid). The rumen fluid was first filtered, than the feed particles and infusoria were separated with centrifugation with 3000 rpm. Subsequently, the rumen bacteria were obtained from the rumen fluid with centrifugation with 16,000 rpm. The obtained rumen bacterium mass was dried with lyophilisation.

The diaminopimelic acid (DAPA) content of the microbial protein mass and of the chymus samples was established with amino acid analyser type Aminochrom-II. The resin fill was Kemochrom 9. The methionine was oxidised into methionine-sulphone with per-formic acid within the sample to be tested to obtain a good separatin of the DAPA (Degussa Analitik/Analysis 1986). The hydrolysis following the oxidation was made with 6 n hydrochloric acid for 24 hours with a partial condenser. The DAPA appears in the place of the methionine, between the valine and the isoleucine.

The discharge of the acid-basis via the urine of the animals was established with the Kutas urine titrating method (Gaál, 1999). The net acid-basis discharge was calculated with the following equation.

NSB = 10 (10 x consumed HCl ml – consumed NaOH ml)

where NSB = net acid basis discharge, mmol/l

3.4. Statistic evaluation of the results

The statistic evaluation of the results of the experiments was made with Statistica 6.0 and Microsoft Excel programs. During the calculations the significance of correlations between the results of the control and experimental phases and groups was studied with the help of the t-probe.

4. New scientific results

The following new scientific results can be summarised on the basis of *in situ*, mobile bag or *in vivo* metabolism tests and of a large-scale milk production experiment made on steers with cannula in the rumen or in the rumen and duodenum:

1. It has been established that the treatment of the extracted soybean meal with hydrochloric acid reduces the degradability of the soy protein in the rumen. The protein degradability reducing effect can be increased via the elevation of the concentration of the hydrochloric acid. Treatment with 10% hydrochloric acid may be recommended from practical reasons (it is technically easier to add a greater fluid volume to the feed in a homogeneous state, and the danger of accident is less). Treatment of 1 kg extracted soybean meal with 200 ml 10% hydrochloric acid (21 g hydrochloric acid/kg extracted soybean meal) reduces the degradability of protein in the rumen significantly in comparison to the untreated soybean meal, relatively by 27.1%.
2. The effect of the hydrochloric acid reducing the protein degradability in the rumen may be increased with heat treatment at 100°C for 30 minutes. As a result of the treatment with acid and of the subsequent heat treatment (combined treatment) the effective degradability of the protein of the extracted soybean meal in the rumen may be significantly reduced in comparison to the untreated

soybean meal, relatively by 63.1%. The combined treatment does not affect the post-ruminal digestibility of the soy protein.

3. Heat treatment made at 100°C for 30 minutes may be replaced with the extraction of the soybean meal treated with acid. In this case it is advisable to add 30% maize to the soybean meal treated with hydrochloric acid for a better extractability. During the extraction of the mixture of 70% soybean meal treated with hydrochloric acid + 30% maize the temperature rises to 150°C in the extractor which is able, together with the generated high pressure, to replace the long-lasting heat treatment.
4. Feeding with extracted soybean meal made with the combined treatment in 1.5 kg daily doses does not notably affect the pH-value, short-chain fatty acid and ammonia contents of the rumen fluid, and does not change the microbial activity of the rumen fluid either. Feeding with the product in the above dose does not reduce the microbe protein volume synthesising in the rumen. At the same time the volume of protein entering the duodenum increases as a result of the effect of the combined treatment reducing the protein degradability in the rumen.
5. Feeding with the product made with the combined treatment shifts the acid-basis balance of the cows towards acid. However, this effect may be efficiently prevented

with buffers added to the feed (e.g. with the addition of 30g $NaHCO_3$ and 40g $CaCO_3$ to 1.5 kg product).

6. Due to the more favourable metabolising protein supply feeding with daily dose of 1.5 kg extracted soybean meal made with the combined treatment increased the daily milk production of the cows significantly by ca. 1 litre. The composition of the milk as well as the amount of nutrients generated in the milk were not affected by feeding with the product.

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

1. Szokoly, Zs., Schmidt, J. (2003): Geschützte Proteine in der Fütterung der Hochleistungskühe, Acta Agronomica Óváriensis, 45 (2): 233-250.

2. Szokoly, Zs., Schmidt, J. Senkung der Abbaurate des Sojaproteins im Pansen mit Salzsäure durchgeführte Behandlung, Megjelenés alatt (Acta Agronomica Óváriensis)

3. Szokoly, Zs., Schmidt, J. (2005): Kombinált kezelés hatása az extrahált szójadara fehérjéjének bendőbeli lebomlására és posztruminális emészthetőségére, Állattenyésztés és Takarmányozás, 54 (4): 339-351.

4. Szokoly, Zs. (2003): Növényi eredetű fehérjék bendőbeli lebonthatóságának csökkentése, IX. IFJÚSÁGI TUDOMÁNYOS FÓRUM, Veszprémi Egyetem, Keszthely

5. Szokoly, Zs. (2004): Sósavval végzett kezelés hatása az extrahált szójadara fehérje bendőbeli lebonthatóságára, XXX. ÓVÁRI TUDOMÁNYOS NAPOK, 94.