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NÖVÉNYOLAJIPARI MELLÉKTERMÉKBŐL ELŐÁLLÍTOTT VÉDETT ZSÍR (Ca-SZAPPAN) FELHASZNÁLÁSA A KÉRŐDZŐK TAKARMÁNYOZÁSÁBAN

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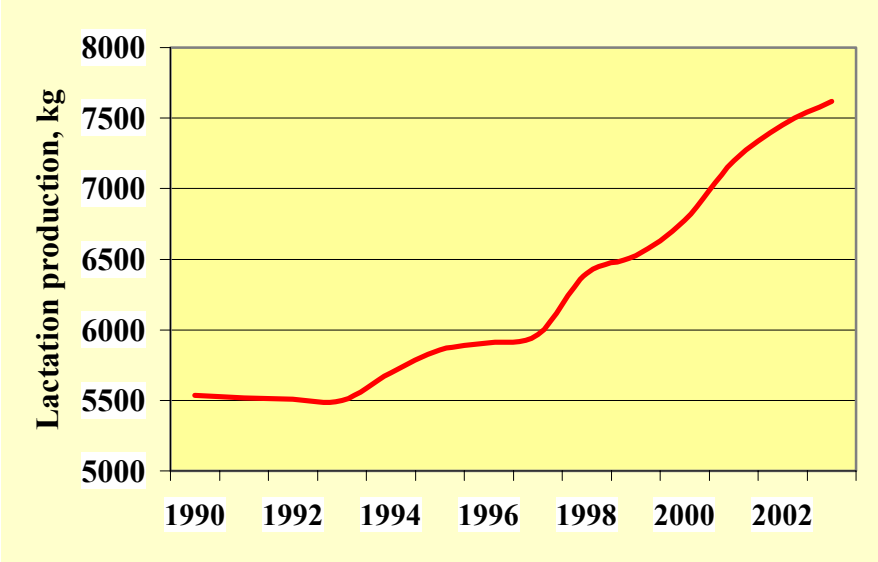
1. INTRODUCTION

Lactation production of cows has significantly increased during the recent years due to the determined work of breeders, to the more and more successful selection procedures and to the application of modern biotechnological methods (figure 1).

1. figure

Lactation production of cow stock examined within the official milk production control in Hungary, between 1990 and 2003 *



** Made on the basis of figures of the OMMI – ÁT Kft. (Gödöllő).*

In certain countries stocks with lactation production between 10,000 and 11,000 kg are quite frequent. Such a large-scale production significantly increases the energy and protein needs of animals and has an extraordinary load on the metabolism.

The most critical period of the feeding of dairy cows is 2 to 3 months following the parturition when the energy balance of the animals is usually negative. This is explained by the fact that the animals' uptake of dry matters does not increase after the post-partum period to an extent which would be demanded by the increase of milk production. The reason is that the milk production reaches the maximum in lactation week 5 or 6 – somewhat later in case of cows performing the first lactation, in lactation week 6 to 8 -, however, the uptake of dry matters culminates only in week 10 to 12 of the lactation. Due to the above phase shift energy deficit occurs in the first week of lactation which the cow tries to compensate by degrading its adipose tissue (Ivings and et al., 1993). According to the studies of Gibb and Ivings (1993) the cows have to degrade 15 to 60 kg of adipose – depending on the extent of energy deficit – in the first two months of the lactation in order to cover their energy needs. The resulting increased adipose mobilisation and the subclinical adipose mobilisation disease imply the risk of development of ketosis. The animals involved do not reach their genetically determined peak production after the post-partum period (Brydl, 1990), and the date of the next successful fertilisation also delays (Haraszti, 1990).

In order to avoid the above detrimental effect the aim is to decrease the daily body weight reduction of the cows below 1.0-1.5 kg, and the total reduction should not exceed 60 kg (Brydl, 2000). This could be obtained via the use of fodders with high energy concentration – at least 6.8-7.0 MJ NE_l/kg. However, the energy supply of the animals cannot be unlimitedly improved by increasing the proportion of provender. The reason is that as a precondition of the perfect operation of the rumen at

least 45% of the daily fodder energy must derive from hay (Schmidt, 1995).

Mainly fats are suitable for the replacement of energy deficit, since their energy content is 2.3- to 2.5-fold higher than that of other feedstuffs. Due to their high energy concentration a great volume of energy enters the organisation without having to extremely increase the dry matter content of the feedstuff.

The significant use of normal (untreated) fats is impossible in the feeding of ruminants, since detrimental consequences derive from feeding with greater volumes of fats. Such detrimental effects could be the impairment of the raw fibre digestion, and consequently the narrowing of the acetate-propionate ratio of the rumen fluid, the reduction of feed consumption. Consequently, the fat and protein content of the milk decreases, and the energy utilization impairs.

The above disadvantages of the fat supplementation may be prevented, or may be significantly reduced via feeding with so-called protected fat products.

2. OWN STUDIES

2.1. Objectives of the experiments

In consideration of the increasing role of protected fat products in the feeding of dairy cows I wished to determine the followings during my experiments:

- How can the Tilley – Terry (1963) *in vitro* procedure widely used for the study of microbial degrading processes taking place in the rumen made suitable for the examination of the degradation of Ca-soaps in the rumen?

- What is the effect of the chain length of fatty acids and of their saturated or unsaturated conditions on the stability of Ca-soaps made from them within the rumen?

- Does the production technology of the Ca-soap influence the stability of the finished product within the rumen?

- What would be the stability of Ca-soaps made from sunflower fatty acid extraction issued as a by-product of the vegetable oil industry within the rumen?

- To what extent could be decreased the negative effects of fat feeding on the functioning of rumen with the product obtained via the saponification of the sunflower fatty acid extraction in comparison to the effect of the untreated vegetable oil?

- Does feeding the Ca-soaps with high unsaturated fatty acid content in doses higher than the usual influence the microbial fermentation in the rumen and the rate of degradation of the crude fibre in the rumen?

- Can Ca-soap made from sunflower fatty acid extraction be used in the feeding of dairy cows, for the supplementation of energy deficit arising during lactation?

- What is the effect of feeding with the product on the fat, protein and lactose contents of the milk?

- Does feeding with the product affect the fatty acid composition of the milk fat and thereby the nutrition value of the milk and of the products made from it?

- Is it possible to increase the linolenic acid (ω-3) content of the milk fat and thus the linoleic acid – linolenic acid ratio essential from nutrition-physiological aspects via feeding with Ca-soap with high linolenic acid content?

2.2. Material and method

2.2.1. Study of stability of Ca-soaps with different fatty acid compositions within the rumen with an *in situ* method

12 cm x 6 cm bags used for the study were made from Scrynel plastic texture the pore size of which was 40 micron. 2 g of Ca-soaps (Profat, Profat with modified fatty acid composition, Ca-soap made from sunflower fatty acid extraction) was dosed per bags, thus the volume of material per 1 cm^2 of the surface of the bag was 13.9 mg. Bags containing the samples were tied to an iron ballast with a weight of 600 g, in order to ensure the bags were submerged in the rumen fluid phase. The ballast was tied to the rumen cannula with a plastic string.

The incubation time was 0, 2, 4, 8, 16, 24 and 48 hours. Each product was tested by animal and by incubation time in 5 repetitions.

The bags were washed on a shaker for 8 x 10 minutes after the incubation. Water used for washing was replaced with fresh one at each of the 8 occasions. After washing the sacks were dried in a thermostat of 60°C.

The effective stability of the examined Ca-soaps within the rumen was calculated with the following equation of Kristensen et al. (1982) on the basis of the measured weight losses:

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

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

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

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

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

During the calculation we presumed that 8% of the rumen content leaves the rumen hourly (k_r = 8%). Naturally, during the use of the above equation protein values were replaced with the adequate fat values.

2.2.2. Study of the stability of Ca-soaps with different fatty acid compositions and made with different technologies within the rumen with an *in vitro* method

Ca-soaps were made from 3 fatty acid mixtures with different compositions (palm seed fatty acids, mixture of various vegetable fatty acids, mixture of artificial fatty acids) for the studies. The mixture of vegetable fatty acids contained sunflower, rape and linseed fatty acid mixture. The mixture of artificial fatty acids was made from the mixture of vegetable fatty acids with the addition of palmitic acid and stearic acid. Fatty acid sources to be used for the soap making were selected and were mixed in a ratio which ensured that not only the fatty acid contents of the Ca-soaps to be made but also their average chain lengths were typically different.

Ca-soaps were made from all the three fatty acid mixtures with two methods – with a one- and two-step technology.

During the one-step technology we had the fatty acids react with $Ca(OH)_2$ at a temperature around 100°C. The ready soap did not require drying.

In the first phase of the two-step procedure fatty acid-Na salts were generated, then - in the second phase - they were converted into Ca-soaps with $CaCl_2$ at 50-60°C. The ready soap was dried at 60°C.

During the *in situ* testing procedure it is a problem that the majority of fatty acids deriving from the hydrolysis of Ca-soaps does not leave the bags in each case. Testing of Ca-soaps with high unsaturated fatty acid ratio involves particularly many problems. The reason is that the unsaturated fatty acids released from the soap are oil-like fluids at the temperature of the rumen, thus they block the pores of the bags by adhering to its inner surface. Fatty acids remaining in the bags – since they are not water-soluble – cannot be completely removed with several water washings following the incubation.

Due to the above disadvantages of the *in situ* procedure an *in vitro* procedure suitable for the determination of the degradability of the fats within the rumen had to be worked out. The development work was based on the *in vitro* procedure of Tilley and Terry (1963) and on its modified version made by Teveli (1977). The essence of the *in vitro* method of Tilley and Terry (1963) and of Teveli (1977, 1978) is that the degradability of fodders within the rumen is determined with the use of inoculum taken from the rumen, in a thermostat of 38-39°C, in the presence of a buffer, via incubation of 48 hours. 4 parts of buffer is used for 1 part of rumen fluid by volume.

The composition of the buffer solution used by us was as follows:

KH_2PO_4	45.36 g/l
NaCl	4.59 g/l
$CaCl_2$	0.20 g/l
$MgCl_2$	0.30 g/l

The pH-value of the solution was set to 6.75 with 10 M NaOH before filling up to the final volume, and - in another experiment - to 6.25. The buffer made in this way was heated to 38-40°C in a water bath, and meanwhile CO_2 was bubbled through it for 30 minutes in order to remove air. The gas flow was ca. 200 bubbles/minute.

The rumen fluid was added to the buffer solution after filtering. CO_2 was bubbled through the inoculum-buffer mixture for further 10 minutes, then the pH was set back to the desired value (6.75 and 6.25) with 10 M NaOH.

The Ca soaps does not provide energy and N for the rumen microbes, therefore the tested products were supplemented with readily fermenting hydrocarbon and N-source. 2 g of Ca-soap was measured by flask (100 ml, narrow-necked Erlenmeyer) to which 0.2 g of $(NH_4)_2SO_4$ and 0,4 g of glucose were added. 50 ml of the inoculum-buffer mixture was used per sample. CO_2 cushion was layered above the fluid before closing the flask. The method of closing made it possible to remove fermentation gases from the flask. The samples were manually shaken 3 or 4 times a day during the incubation. The temperature of the thermostat was 40°C. Each treatment was repeated 10 times.

After the incubation period (48 hours) the fermentation was stopped with the addition of 0.5 ml formaldehyde solution of 35% in each flask.

Subsequently, the contents of the flasks were poured into culture dishes, than they were dried at 60°C.

The volume of fatty acids released from the Ca-soaps was determined via dissolution with acetone after drying. The results were corrected with the fat content of the rumen fluid as well as with the "free" fat content of the Ca-soap (which can be dissolved from the tested soap also without incubation). The fat content of the rumen fluid was determined from blind samples without Ca-soap.

2.2.3. Digestion-physiological basis studies with rumen and duodenum canulated steers

2.2.3.1. Effect of different fat sources on the composition of the rumen fluid and on its microbial activity

The effect of fats on the rumen fermentation was tested in three experiments. In the first experiment the effects of fats with different chemical forms on the rumen functioning were compared. During the study first Ca-soap made from sunflower fatty acid extraction was given to the animals, subsequently the same fat volume was fed in the form of untreated sunflower seed oil - in order to confirm the stability of the soap within the rumen.

In the second experiment the effects of Ca-soaps with different fatty acid compositions (Profat, Profat with modified fatty acid composition and Ca-soap made from sunflower fatty acid extraction) on the rumen fermentation were compared. Two of the three tested Ca-soaps (Profat and soap made from sunflower fatty acid extraction) were significantly different from each other in respect of the unsaturated fatty acid content.

During the third experiment the effect of Ca-soap with high unsaturated fatty acid content on certain parameters of the rumen fluid was examined. Ca-soap used for the experiment was made from a basic material containing 80% linseed oil and 20% flax fatty acid mixture.

The experiments were made with 3 rumen and duodenum canulated steers, with a batch method. All the experiments were repeated once. All the three experiments included also a control phase when no fat supplementation was fed to the animals. In addition to the control feed dose the tested fat supplementation was fed in two parts a day, via cannulas to the rumens of the animals. Thereby the possibility that the animal leaves a part of the product was excluded, which would disturb the evaluation of the experiment. The supplementation was 800 g a day in case of each Ca-soap. Ca-soap used in the first experiment contained 80% crude fat, thus the animals consumed 640 g crude fat a day via the Ca-soap. Consequently, 640 g sunflower seed oil was fed in the next phase of the experiment. The oil was mixed into the daily feed ration, and the oily concentrate mixture was given to the animals also via the rumen cannula.

The period of the control and of the experimental phases was 4 days. A 10-day transitional period was kept between the 4-day experimental phases. The animals were gradually accustomed to the fat types fed to them via transitional periods.

Samples were taken from the rumen fluid on the four days of the control and experimental phases, on 2 occasions a day – before the morning feeding and 3 hours after the feeding. The samples were delivered to the laboratory in a thermos and their testing was immediately

started in order to avoid loss of microbial activity in the rumen fluid. The following parameters of the rumen fluid were specified: pH-value, NH_3-content, microbial activity and the concentration of the individual volatile fatty acids. Test results of samples taken before and after feeding were separately processed.

2.2.3.2. Effect of fats on degradation of the crude fibre in the rumen

The effect of fat supplementation on the degradation of the crude fibre in the rumen was tested within the first and third experiments, on 3 animals with cannula in the rumen and duodenum. Chymus samples were also taken from the animals – in addition to the rumen fluid – for the tests. The chymus was collected on days 2 and 4 of the 4-day testing phases. Samples were taken with two-hour intervals between 6.00 a.m. and 4.00 p.m. on the collection days. The chymus was dried at 60°C in the laboratory for the performance of the adequate chemical tests (crude fibre content, titan content).

The daily chymus volume passing through the duodenum was specified with the use of an indicating agent, with the method of Owens and Hanson (1992). 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 - irrespective of the feed uptake - the TiO_2 was fed into the rumen directly via the cannula in 2 parts (2x30 g) a day.

2.2.4. Methodology of the field trials

The aim of our first field trial was to increase the unsaturated fatty acid content of the milk fat via feeding with Ca-soap with high unsaturated fatty acid content, with a special regard to the ω-3 fatty acids. In addition, we studied the effect of feeding with the product on the fat, protein and lactose contents of the milk.

Ca-soap used for the experiment was made from 80% linseed oil and 20% flax fatty acid mixture, and consequently, more than 50% of its fatty acid content was constituted by linolenic acid belonging to the ω-3 group.

The experiment was made with a group method. 21 Holstein-Friesian cows were involved in both the control and the experimental group. The composition of the milk was almost identical at the beginning of the experiment in respect of the average of the groups.

The cows of the experimental group consumed 700 g Ca-soap a day via the dairy concentrate. Their energy and metabolising protein supply was similar to that of the control animals. The transition period was 2 weeks, and the experimental period was 5 weeks.

The cows were milked 3 times a day. During the experimental phase individual samples were taken at the morning and evening milking on 2 days a week in order to specify the composition of the milk. Samples to be tested were made from the partial samples taken in the morning and in the evening by mixing 60% (morning partial unit) and 40% (evening partial unit) in each case. Samples were taken for the testing of the fatty acid composition of the milk fat 3 times a week from the mixed milk of the two groups, at the evening milking. The composition of the milk

samples was tested by the Állattenyésztési Teljesítményvizsgáló Kft. (Gödöllő) on its automatic device type System 5000 (manufactured by the Foss Electric, Hillerod, Denmark), during which the fat, protein and lactose contents of the milk were specified.

During our second field trial the effect of Ca-soap made from a by-product of the vegetable oil industry (sunflower fatty acid extraction) on the milk production and on the milk composition was tested.

15 multiparous Holstein-Friesian cows were involved in the experiment, which performed their 3rd lactation in general. The average milk production of the group was 8442 litre in the previous lactation, and it was day 55 of the current lactation at the starting of the experiment.

The experiment was made with a batch method. Both the control and the experimental phases took 4 weeks. In order to prevent the reduction of production deriving from the progress of the lactation from disturbing the comparability of the results the control phase was divided, i.e. two weeks were applied before the experimental phase and two after it. During the evaluation of the experiments the results of the two two-week control phases were combined, and the average of the 4 control weeks was compared to the average of the 4 experimental weeks. One-week transitional period was applied between the control and the experimental phases.

During the experimental phase the cows consumed 700 g Ca-soap a day via the dairy concentrate. Their energy and metabolising protein supply was the same as in the control phase.

The animals were milked twice a day. Their milk production was individually measured via 5 days a week. Individual 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 Kft. (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 (manufactured by the Foss Electric, Hillerod, Denmark).

2.2.5. Chemical testing procedures used during the experiments.

The chemical composition of the feeds, the Ca-soaps and of the chymus samples (dry matter, crude protein, crude fat, crude fibre, crude ash, Ca, P) was specified with procedures recommended in volume 2 of the Hungarian Alimentary Code (1990). The crude fat content of the Ca-soaps was calculated on the basis of the dry matter and crude ash contents.

The pH-value of the rumen fluid was specified with an electronic pH-meter type OP-211/1, and its NH_3 content with an ammonia-sensitive electrode type OP-264/2. The microbial activity of the rumen fluid was examined with a nitrite reduction test, 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). Alpha-naphthyl-amine was used as a reagent (Horváth, 1979). The volatile fatty acid content of the rumen fluid was specified with a gas-chromatographic equipment type Chrom-5. Before testing the rumen fluid was cleaned via centrifugation at 15,000 rpm and via filtering, then

it was treated with 25% metaphosphoric acid before the injection. The column charge of the gas-chromatographic equipment was Porapak P resin. The water standard solution used for the identification contained volatile fatty acids with a concentration of 0.1.

The fatty acid contents of the fed fat supplementations were specified with gas-chromatographic equipment type Chrom-5, with Chromosorbe W AW column charge in the *in vivo* model experiments, and with gas-chromatographic equipment type Agilent 6890N Network, with Supelco SP^{TM} – 2560 Fused Silica Capillary column in the plant experiments. The fatty acid composition of the milk fat was specified also with the latter device.

Sunflower oil was esterified with a 75:25:4 mixture of methanol, benzene and sulphuric acid for the testing to be made on the device type Chrom-5 (MSZ 19928-73). Subsequently the samples were washed into a shaking hopper with a 1:1 mixture of diethyl-ether and petroleum ether, then were disacidified with saturated NaCl solution. At the testing of Ca-soaps the fatty acids were released via boiling in a 5% HCl solution. Subsequently the preparation of the sample was identical to that of the sunflower seed oil.

The pure fats were saponified with 1 n methanol-containing NaOH solution, and then were treated with BF_3 methanol for the testing made with device type Agilent 6890N Network. No saponification was made in case of Ca-soaps.

The prepared test materials were solved in n-hexane in both cases for the injection. Standard made for the identification of the fatty acid contained fatty acid-methyl-esters in a hexane solution.

The titan content of the chymus was specified with spectrophotometer 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.

2.2.6. Statistic evaluation of the results

The statistic evaluation of the results of the experiments was made with Statistica 6.0 and Microsoft Excel programs.

3. NEW SCIENTIFIC RESULTS

On the basis of the laboratory measurements, *in situ* and *in vitro* tests, model experiments made rumen and duodenum canulated steers as well as of the results of the field trials the following new scientific results may be established:

1. A new testing procedure has been worked out via the development of the *in vitro* method of Tilley and Terry (1963) which is suitable for the testing of degradation of Ca-soaps in the rumen. The essence of the modification of the basic procedure is that nutrients, namely $(NH_4)_2SO_4$ and glucose are provided for the rumen microbes, and that the pH-value of the fermentation agent is set to a value (6.75-6.25) lower than the optimum (6.9-7.2). The stability of certain Ca-soaps within the rumen - in particular, in case of products with high unsaturated fatty acid ratio - may be assessed with the improved method more reliably than with the *in situ* method.

2. It has been established that beyond the unsaturation of the fatty acids their chain length also significantly influences the stability of Ca-soaps made from them within the rumen, i.e. Ca-soaps of fatty acids with a short chain length have weaker stability in the rumen. The reduction of stability within the rumen may be observed already with a higher saturated fatty acid content in case of Ca-soaps with C_{14-15} average chain length.

3. The production technology of the Ca-soap also affects the stability of the product in the rumen. In case of similar fatty acid compositions Ca-soap

made with the one-step production technology - due to its different physical properties - is more stable in the rumen circumstances than products made with the two-step method.

4. Ca-soap with sufficient stability in rumen may be made from sunflower fatty acid extraction generating as a by-product of the vegetable oil industry, which disturbs the rumen fermentation to a slight extent only even in doses higher than the average (800 g/day). Feeding with the product has no actual effect on the degradation of the crude fibre in the rumen. Ca-soap made from sunflower fatty acid extraction may be used in the feeding of dairy cows with good results, for the supplementation of energy deficit arising during lactation. Feeding with the product has a beneficial effect on the fatty acid composition of the milk fat, since it increases the ratio of the unsaturated fatty acids in the milk fat to the debit of saturated fatty acids with average chain length.

5. Via feeding with Ca-soap with high linolenic acid content the linolenic acid (ω-3) content of the milk fat may be doubled. Consequently, the linoleic acid – linolenic acid ratio decreases in the milk which has a very beneficial effect from human nutrition and physiological aspects. In case of feeding with such a soap the appearance of trans-fatty acids may be expected in the milk fat which impairs the nutrition-biological value of the milk on one part, and decreases its fat content on the other part.

4. LIST OF PUBLICATIONS MADE IN THE THEME OF THE ESSAY

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2. Ribács A. (2002): Növényolajipari melléktermékből előállított Ca-szappan hatása a bendőfermentációra. 13. Magyar Buiatrikus Kongresszus, Hajdúszoboszló, 10th-12th Oct. 31-36.

3. Ribács A. - Schmidt J. (2003): Növényolajipari melléktermékből előállított Ca-szappan hatása a bendőfermentációra. Állattenyésztés és Takarmányozás, 52 (6) 567-579.

4. Ribács A. (2003): Különböző zsírsav-összetételű Ca-szappanok hatása a bendőműködésre. 14. Magyar Buiatrikus Kongresszus, Keszthely, 9th – 11th Oct., 106-114.

5. Ribács A. - Schmidt J. (2004): Különböző kémiai formájú zsírok hatása a nyersrost bendőbeli lebomlására. XXX. Óvári Tudományos Napok, Mosonmagyaróvár, 7th Oct., 90.

6. Ribács A. (2005): Növényolajipari melléktermékből előállított Ca-szappan felhasználása tejelő tehenek takarmányozásában. Állattenyésztés és Takarmányozás, 54 (2) 159-170.

7. Ribács, A. - Schmidt, J. (2005): Einfluss von Fetten mit unterschiedlicher chemischer Form auf den Abbau der Rohfaser im Pansen und auf einige Parameter der Pansenflüssigkeit. Acta Agronomica Óváriensis, Megjelenés alatt.