THESIS OF DOCTORAL (PhD) DISSERTATION

VISZKET ERNA

MOSONMAGYARÓVÁR

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IMPROVING N-3 FATTY ACID CONTENT OF BOVINE MILK BY FEEDING

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1. INTRODUCTION

Milk has special role in the human nutrition for more aspect (calcium, protein, vitamin and mineral content). In spite of these facts, milk has got lot of negative criticisms, which claim that the higher cholesterol and saturated fatty acid content of the milk are responsible for several cardiovascular diseases, for example: arteriosclerosis, high blood pressure, high risk of heart attack.

According to the published results the fat sources and products (for example full-fat seeds, Ca-soaps, hydrogenated fats, oils etc.) applied in the dairy cows diet can influence not only the milk production but also the nutrient content and the fatty acid profile of the milk fat (*Komprda* et al., 2005; *Ribács* and *Schmidt*, 2006; *Várhegyi* et al., 2007; *Kudrna* és *Marounek*, 2008; *Murphy* et al., 2008). Among oil resources fish oil may be suitable for improving the fatty acid composition of milk. Its reason is that fish oils contain n-3 fatty acids (eikozapentaenic acid, EPA, C20:5; docozahexaenic acid, DHA, C22:6) in high concentration, which have beneficial effects for human health. Dietary n-3 polyunsaturated fatty acids have effects on diverse physiological processes, such as the regulation of plasma lipid levels, cardiovascular and immune function, and neuronal development and visual function (*Jump*, 2002). The other group of long chain polyunsaturated fatty acids is the group of n-6 fatty acids (for example: linoleic acid, LA, C18:2). While the optimal n-6/n-3 ratio is 4:1 (or narrower) in the European and American types of diet this ratio may reach the rate of 10:1 or 30:1 for the daily fatty acid consumption. To solve this problem it may be important to develop such foods, which contain the n-3 fatty acids in higher

proportion. One possible solution should be to produce milk and milk products rich in n-3 fatty acids, because these foods are integrant part of the daily nutrition.

One of the most important aims of my PhD dissertation is to search such feeding methods, which can influence the fatty acid composition of milk fat beneficially, with especial regard to the n-3 fatty acids and conjugated linoleic acids (CLA).

2. OWN EXPERIMENTS

2.1. The aim of the experiments

In recent years numerous experiments were carried out to increase the n-3 fatty acid content in some animal originated food (e.g. milk, egg and meat) at the University of West Hungary, Faculty of Agricultural and Food Sciences (UWH-FAFS), Department of Animal Nutrition.

Main aims of our experiments were to investigate the effect of a fish oil based bypass fat supplement and the utilisation of grass haylage based diet on the milk production of dairy cows and nutrient content (e.g. protein, fat), fatty acid profile and organoleptic properties of the milk.

Therefore, the following questions were asked:

- What protein and fat contents and fatty acid composition could the raw bovine milk samples have delivered to a cheese factory located in the western part of Hungary, based on the results of a 2-year-long trial?

- What would be the stability of the fish oil based fat product developed by a coating technology in the rumen?

- How could the fish oil based fat product feeding influence the major properties (pH, ammonia, volatile fatty acid content, microbial activity) of rumen fermentation?

- What kind of effect could the fish oil based coated fat product have on the nutrient content and fatty acid profile of the milk, utilising it alone or combining it with grass haylage based diet?

- How could the feed ration, which can be considered novel in Hungary (combined utilisation of grass haylage based diet and fish oil based product) influence, the major sense (organoleptic) properties (e.g. taste, flavour) of the milk?

2.2. Materials and methods

2.2.1. Evaluation of the seasonal effects on the concentrations of fat and protein, and on fatty acid profile of the milk produced on dairy farms located in the western part of Hungary

A 2-year-long survey was carried out to determine the nutrient content and fatty acid composition of the milk produced on Hungarian dairy farms applying all-year-round use of diets. Besides, the effects of the seasons on the parameters mentioned above were investigated. Samples were collected at weekly intervals at Óvártej Zrt. in Mosonmagyaróvár, where approximately 150,000-180,000 litres of milk arrive from 35-40 dairy farms daily. The studies were conducted between 1st September 2008 and 31th August 2010. The length of experimental periods was 2×3 months (spring, summer, fall, winter). We have not got available information about the feeding technology of the farms produced the examined milk.

The protein and fat contents and the fatty acid composition of the milk samples were determined.

2.2.2. Model experiments with rumen canulated steers

2.2.2.1. Evaluation of the rumen stability of the bypass omega-3 fat product (product 1.) by *in situ* method

The ruminal degradability of the crude fat content of the developed omega-3 fat product (produced by Adexgo Ltd., Balatonfüred, Hungary) and that of a commercial hydrogenated fat (Hidropalm, Norel S.A., Spain) was investigated with 4 Holstein-Friesian steers weighing 600 kg using *in situ* method.

The 5 bags used for the trial were made from Scrynel plastic with a pore size of 40 micron, their size were 12×6cm. 2 g of experimental samples were dosed per bag, thus the volume of material per 1 cm^2 of the surface of the bag was 13.9 mg. The length of incubation times (hour) were as follows: 0, 2, 4, 8, 16, 24 and 48 in the trial. Each product was tested by animal and by incubation time in 3 replications.

Bags were washed for 3×10 minutes after the end of the incubation periods. Water used for washing was changed to clear water in all three cases. Bags were dried at 60°C after washing.

The actual rumen stability of the products (omega-3 fat product, Hidropalm) was calculated on the basis of dry matter losses using the equation of *Kristensen et al.* (1982). During the calculation the rumen passage (outflow) rate was 0.08/h (kr =8%).

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

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

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

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

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

The protein values were replaced of course with the adequate fat values in the equation written above.

2.2.2.2. The effect of omega-3 product (product 1) utilisation on several properties of the rumen fermentation

The experiment was carried with 4 rumen canulated steers. The control and the experimental periods (1 and 2) were performed in two replications. No fat supplement was added to the animal's diet in the control period, however, 0.25 kg/animal/day (2.2% of dry matter intake) omega-3 product was given to the steers in the 1st, and the same amount of Hidropalm in the 2nd experimental period. In the 5-day test phase, after a 10-day adaptation period, rumen fluid samples were taken through the fistulae four times a day (before the morning feeding and 2, 4, and 6 hours after the feeding) and the following parameters were analysed: pH, NH_3 and SCFA content (acetate, propionate, butyrate, etc.), and rumen microbial activity.

2.2.2.3. Evaluation of the rumen stability of the improved omega-3 product (product 2) by *in situ* method

The rumen stability of the improved omega-3 product (product 2, name of the product: Adexgo-1, producer: Adexgo Ltd., Balatonfüred) was

determined within the frame of another model experiment. The analysis was performed in the same way as it was written in *chapter 2.2.2.1.*

2.2.2.4. The effect of improved omega-3 product (product 2) utilisation on several properties of the rumen fermentation

The effect of improved omega-3 product mentioned above, on the rumen fermentation was also evaluated. This experiment was carried out with 4 rumen canulated steers (Holstein-Friesian). The analysis was performed in the same way as it was written in *chapter 2.2.2.2.*

2.2.3. Methodology of the farm trial

The effect of both omega-3 fat products on the milk production, the amount of nutrient content of daily produced milk and the fatty acid composition of the milk were assayed during farm trials. These experiments were conducted with Holstein-Friesian cows on the dairy farm of Solum Zrt. in Komárom.

2.2.3.1. Preliminary farm trial ('Pre-examination')

A preliminary farm trial was conducted to evaluate the influence of the corn silage based diet supplemented with low dose (0.25 kg/animal/day) of omega-3 fat product (product 1) on the milk production, the nutrient content and fatty acid profile of the milk. The experiment involved 25 multiparous cows, which have usually completed 2nd or 3rd lactation and were at the 3rd part of their current lactation (average daily milk production was 18.69±2.8 kg 2 weeks before the experiment). The trial had two phases: a control and an experimental one. The same 25 cows were used during the whole experiment. The control phase consisted of a 2-week adaptation and a 4-

week test period. After it, in the experimental phase, another 2-week adaptation period started, during which cows were accustomed to the 0.25 kg bypass omega-3 product gradually. Finally, the 4-week test period was carried out.

Considering that the 0.25 kg bypass omega-3 product was not possible to use directly in the mixing-distributer carriage equipped with weighing machine, so a 'pre-mixture' was performed (the omega-3 product was mixed with a part of the corn meal).

The cows are milked twice a day in the dairy farm of Solum Zrt. This farm has a PC-controlled milking system, so the individual milk production of the cows per milking could have been registered every day. The milk composition was analysed from the evening milk individually once a week.

Besides the individual milk samples, bulk milk samples were also collected two times in the control and in the experimental period to determine the fatty acid composition of the milk using gas chromatograph.

2.2.3.2. Farm trial 1

The effect of the grass haylage based diet supplemented with the improved omega-3 product (product 2) was evaluated in a farm trial, as well. 80 multiparous cows were involved in both the control and the experimental groups. They have usually completed 2nd or 3rd lactation and were at the 3rd part of their present lactation (average daily milk production was 21-22 kg 2 weeks before the experiment). The animals in the control group were fed with corn silage-alfalfa haylage-cornmeal based diet, while the diet of the experimental cows based on grass haylage-alfalfa haylage-cornmeal. The dose of the omega-3 fat product (product name: Adexgo 1., producer:

Adexgo Ltd., Balatonfüred) was 0.5 kg/day for the cows in the experimental group. This fat product contained fish oil rich in EPA and DHA, which was protected from the rumen degradation by an own improved coating technology.

The farm trial took 6 weeks long after the 3-week adaptation period. In the adaptation period cows were accustomed to intake the grass haylage based diet and the omega-3 fat product. The milk composition was determined from milk samples of the evening and morning milking individually once a week. Besides the individual milk samples, bulk milk samples were also collected two times during the morning and the evening milking to determine the fatty acid composition of the milk using gas chromatograph.

2.2.3.3. Farm trial 2

The 2nd farm trial was conducted also on the dairy farm of Solum Zrt. in Komárom. The experiment involved 40 multiparous high yielding cows (average daily milk production was 31.2±1.38 kg 2 weeks before the experiment). At the beginning of the experiment cows fed a corn silage-alfalfa haylage-cornmeal based diet, which is commonly used in Hungarian dairy cows nutrition, and then after a 14-day adaptation period the animals started to intake the grass-haylage based diet (control period). This period took 10 weeks long. After it another 14-day adaptation period started and then in the experimental period, cows fed grass haylage based diet supplemented with 0.5 kg of omega-3 product with modified fatty acid composition (product name: Adexgo 1., producer: Adexgo Ltd., Balatonfüred) for 10 weeks. The same 40 cows were involved during the whole farm trial therefore the effect of feeding on the milk production and

milk composition should be advisable to take into consideration only as informing data. However this method allowed us to not take into account the effect of individual differences considering fatty acid profile.

Milk samples were collected from the cows once a week (always on Wednesday), and the milk composition (dry matter, protein, fat and lactose content) was analysed from the evening milk, furthermore the fatty acid profile of the bulk milk was determined. The milk production of the cows was registered per milking every day.

2.2.4. Sensory analyses (Evaluation of organolpetic properties)

Sensory analyses were performed during farm trial 1 and 2 to evaluate the effect of feeding grass haylage based diet and omega-3 fat product, as well as the different pasteurization's temperatures on the organoleptic properties of the milk with improved fatty acid profile. Sensory analysis was organized at the NYmE-MÉK Department of Animal Nutrition three times with 20, 44 and 16 participants, respectively. The members of tasting board consumed pasteurized milk and evaluated the properties (smell, colour and taste) on a 0-5 point scale (0=very bad; 5=excellent).

The colleagues of Campden BRI Magyarország Nonprofit Ltd.(Budapest, Hungary) classified also in an independent study the major characteristics of the milk produced by modified feeding system and a commercial milk product (Tolle tej 2.8%, Tolnatej Ltd., Szekszárd, Hungary). The assessors analysed whether the temperature of pasteurization (72°C or 90°C) could influence the examined properties. In this case the 6 assessors evaluated 14 properties. A 0-9 point scale scoring was performed (0=very bad; 9=excellent).

2.2.5. Chemical analyses (applied in the experiments)

Dry matter, crude protein, ether extract, crude fibre, and crude ash content of diets were measured according to Hungarian National Standard (Hungarian Feed Codex, 2004).

The pH value of rumen liquid samples was measured by electronic pH meter (type: OP-211/1, Radelkis Ltd., Budapest, Hungary) and the NH_3 content of it was determined by ammonium electrode (type: OP-264/2, Radelkis Ltd., Budapest, Hungary). Nitrite reduction test with three different nitrite concentrations (0.2, 0.5, and 0.7 ml of 0.025% KNO2 solution/10 ml rumen fluid) was used to determine the microbial activity in the rumen fluid samples. Alpha-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 (e.g. propionate, acetate) concentration of rumen fluid was measured using a liquid chromatograph (Biotronik, Wissenschaftliche Geräte GmbH, Germany).

The fatty acid composition of the feeds and milk samples was analysed using a gas chromatograph (HP Agilent Technologies 6890N, Foster City, CA, USA). The type of column was Supelco SP™ 2560 Fused Silica Column (Supelco, Bellefonte, PA, USA) 100 m×0.25 mm×0.2 μm film thickness; carrier gas: H.

Lipids extracted from the milk with centrifuging were treated with chloroform/methanol (2:1, vol/vol). The disjunction of the phases was assisted by 0.9% salt solution. After evaporation of solvents, samples were saponified with 1 n NaOH at 100 °C. Boron-trifluorede-methanol was used

for esterification of fatty acids and then the samples were solved in hexane, centrifuged, and dissolved prior to injection.

The fat, protein, lactose and dry matter content of milk samples derived from the preliminary, the farm trial 1 and farm trial 2 were analysed by Hungarian Dairy Research Institute (Mosonmagyaróvár, Hungary) using MilkoScan™ Minor 4 (FossAnalytical, HillerØd, Denmark). The pasteurization, condensation and pulverization of the milk samples in the farm trial 1 were also performed in the Hungarian Dairy Research Institute. Pasteurization was executed with PA plate pasteurizer (Nagema, Germany), condensation was performed with downcomer vacuum booster (Anhydro, Denmark), while Niro pulverizer (Anhydro, Denmark) was used in pulverization.

Protein and fat content of milk samples derived from the dairy farms located in the western part of Hungary were determined by the colleagues of Óvártej Zrt. using MilkoScan™ Minor 4 (FossAnalytical, HillerØd, Denmark).

2.2.6. Statistical analyses

Data were analyzed (*Kolmogorov- Smirnov test, t-test, ANOVA, Kruskal-Wallis test, Mann-Whitney test, correlation analysis*) using the *SPSS 15.0 for Windows* program package (SPSS Inc., Chicago, USA).

The colleagues of Adware Research Ltd. (Balatonfüred, Hungary) assisted in performing statistical evaluation.

3. NEW SCIENTIFIC RESULTS

Based on the results of the investigations the following new scientific results can be stated:

1. For each of the seasons statistically proved differences were found in the fatty acid compositions of bovine bulk milk samples examined during different periods of years (spring, summer, autumn, winter) regarding all the saturated fatty acids (SFA) analyzed – except for heptadecanoic acid (C17:0) – and in respect of linoleic acid (C18:2) and eicosatrienoic acid (C20:3) levels of the polyunsaturated fatty acids (PUFA).

2. According to the results of i*n situ* experiments the omega-3 fat product II developed by a special coating technology showed 70.5% effective fat stability. No unfavourable effect was detected on some parameters of ruminal fermentation (pH, NH_3, short-chain fatty acids, SCFA concentration) when the amount of 2.2% in daily DM intake of omega-3 fat product II was applied.

3. Besides feeding of grass haylage-alfalfa haylage-corn meal based diet the 0.5 kg/day unit of the fish oil based omega-3 product II significantly ($P \leq 0.05$) improved the rate of the n-3 fatty acids and the tested CLA isomers in the milk fat. The experimental diet had no negative effect on the organoleptic properties (smell, taste, colour) of the pasteurized milk samples.

4. In experiments aiming to modify the fatty acid composition of milk longer experimental periods (10 weeks or longer) are suggested to set up, because in the early phase of the trials trans vaccenic acid (t11-C18:1) and elaidic acid (t9-C18:1) appearing in an extremely high concentration showed a gradually decreasing tendency during the experimental period lasting for 10 weeks.

4. LIST OF PUBLICATIONS IN THE THEME OF THE DISSERTATION

Scientific paper published in Hungarian language

1. ***Viszket E.*** *– Zsédely E. – Tanai A. – Varga L. – Tóth T.* (2010): Az évszak hatása a tehéntej zsírsav-összetételére. Tejgazdaság. LXX. 1-2. 15-21.

2. *Tóth T. –* ***Viszket E****. – Csavajda É. – Tanai A. – Fébel H.* (2011): Halolaj alapú védett zsírkészítmény bendőbeli lebomlásának, és a tehenek termelésére és tejösszetételére gyakorolt hatásának vizsgálata. Állattenyésztés és Takarmányozás (*accepted*).

3. *Tóth T. –* ***Viszket E.*** *– Csavajda É. – Tanai A. – Fébel H.* (2011): Fűszenázs alapú takarmányozás és burkolással előállított halolaj alapú zsírkészítmény kombinált etetésének hatása a tej zsírsavösszetételére. Acta Agronomica Óváriensis, 53. 1. 17-33.

4. ***Viszket E****. - Csavajda É. - Varga L.- Tanai A. - Tóth T.* (2011): Adatok a nyugat-magyarországi nyers-tejminták zsírsav összetételére vonatkozóan. Acta Agraria Kaposváriensis (*accepted*)

Scientific paper published in foreign language

1. *Tóth T. –* ***Viszket E.*** *– Csavajda É. – Tanai A. – Varga L.* (2011): Seasonal changes in the fatty acid composition of raw bovine milk produced in the western part of Hungary (2008-2010). Milchwissenschaft – Milk Science International (*accepted*)

Oral peresentations in Hungarian

1. ***Viszket E.*** *– Tóth T.* (2010): Halolaj alapú zsírkészítmény bendőbeli stabilitása és etetésének hatása a bendőfermentáció néhány paraméterére. LII. Georgikon Napok, Keszthely, 2010. szeptember 31-október 1.

2. ***Viszket E****. – Zsédely E. – Tanai A. – Varga L. – Gyurcsó G. – Tóth T.* (2010): A tehéntej zsírsav-összetételének alakulása a nyugat-magyarországi régióban. XXXIII. Óvári Tudományos Nap, Mosonmagyaróvár, 2010. október 7.

3. ***Viszket E***. – *Csavajda É.* – *Varga L.* – *Tanai A.* – *Tóth T*. (2011): Adatok a nyugat-magyarországi nyers-tejminták zsírsav összetételére vonatkozóan. 15. Nemzetközi Takarmányozási Szimpózium, Kaposvár, 2011. június 3.

4. ***Viszket E.*** - *Zsédely E.* - *Tanai A.* - *Varga L.* - *Tóth T*. (2010): Az évszak hatása a tehéntej zsírsav-összetételére. *Takarmányozással Foglalkozó Oktatók és Kutatók Konferenciája.* Az előadások összefoglalói. Szegedi Tudományegyetem Mezőgazdasági Kar, Hódmezővásárhely: *Agrár- és Vidékfejlesztési Szemle* 5 (2), 121–121.