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MILK WITH FUNCTIONAL FOOD PROPERTIES

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ABSTRACT: This paper presents the results reflected on obtaining the milk enriched with omega-3 fatty acids. As source of omega-3 fatty acids, product of French company Vitalac called "TradiLin", was used. Trial was carried out on 20 trials (T) and 20 control (C) cows of Simmental breed and it lasted 80 days. Average content of omega-3 in trial and control group was 0.586 and 0.284 g/100g of extracted fat ($P \leq 0.01$), whereas content of omega-6 in same order of treatments was 3.141 and 3.011 g/100g of extracted fat ($P \leq 0.05$). Ratio omega-6/omega-3 fatty acids of 10.602 (control) was declined to 5.360 (trial), which is considered to be desirable from the aspect of nutrition with milk as functional food. Also, in other milk final product called "mladi kajmak", obtained by taking off milk fat from the surface of heat treated and cooled milk, content of omega-3 in trial and control group showed the same trend, 0.76 and 0.41 g/100g of extracted fat ($P \leq 0.01$), whereas content of omega-6 were 3.90 and 3.79 g/100g of extracted fat ($P \leq 0.05$). Ratio omega-6/omega-3 in control group was reduced of 9.24 to 5.10, which are considered to be extremely favourable. Chemical constituents of milk, somatic cell count as well as total plate count showed no differences between groups ($P > 0.05$). Also, investigated treatment had no effect on changes in biochemical blood parameters.

Key words: *milk, omega-3 fatty acids, functional food*

INTRODUCTION

The term "functional food" includes those food stuffs which beside their main nutritive value contain such substances which have impact on improvement of general health condition, i.e. they have preventive and therapy effect (Sretenović, 2005).

In this trial as a source of omega-3 fatty acid, product known under the trade name "TradiLin", is used. The varieties of flax are specially selected for their richness in ALA omega-3 fatty acids. "TradiLin" is produced from a fully patented extrusion technology which liberates the oils without denaturing them, so they can be digested more rapidly in such a way that it is available to the animal at the right time and at the right dose, inactivates the anti-nutritional factors (cyanogens) and preserves the fatty acid profile.

Typically 60% of fat intake for an average human comes from animal products (meat, milk, eggs, etc). These animal products have changed because the feeds that the animals now eat have changed. In the past times 40 – 50 years ago, animals used to eat mainly forage (high omega-3, lower omega-6) but now typically eat less forage but consume more cereals and soya (high omega-6, low omega-3) and as a result of that animal products (meat, milk, eggs etc) are much higher in omega-6 and lower in omega-3. So now the human diet, because of what animals are fed, is higher in omega-6 and lower in omega-3 i.e. traditional diets gave an omega-6: omega-3 ratio of 1:1, current human diets show a ratio of 20:1 and in animal diets up to 40:1.

Scientific studies suggest that this imbalance of omega-6:omega-3 ratios is the cause of numerous physiological disorders and that better-balanced ratios could result in significant health improvements (Association Bleu-Blanc-Coeur, 2004).

In order to overcome numerous health problems, the food stuffs which aren't traditional sources of omega-3 fatty acids such as dairy products and pastry, meat, baby foods, etc. are enriching with moderate quantities of these fatty acids and there is increased demand for such products because of their well known positive effect on human health (Sretenović *et al.*, 2007a).

Since human organism has no enzymatic system necessary for synthesis of omega-3 fatty acids, they must be introduced through diet (they are called “essential fatty acids”).

Modern biotechnologies offer different ways for enriching of products with omega-3 (Sretenović *et al.*, 2007, Sretenović *et al.*, 2009, Sretenović *et al.*, 2009a). Products of animal origin such as milk, meat and eggs, enriched with omega-3 fatty acids are obtained by inclusion of sources of these acids into animal diets. Also, increase of the content of omega-3 fatty acids is achieved by application of modern biotechnological procedures in selection and plant breeding, by growing of varieties which synthesize higher quantity of ALA, i.e. fatty acids similar to EPA and DHA.

In connection with the above mentioned, goal of this research was to get milk and dairy products enriched with omega-3 fatty acids through the introduction of the “TradiLin” preparation in diets of lactating cows.

MATERIAL AND METHODS

Trial was carried out on 40 cows of Simmental breed, divided into two groups with 20 cows in each one. Both groups were made uniform according to relevant criteria, i.e. they were in the second lactation and with similar quantity of milk in previous lactation.

Source of omega-3 fatty acids was product “TradiLin” representing special type of flax processed using specific patented technological procedure.

In the laboratory of the Institute for Animal Husbandry the chemical composition of the product “TradiLin” was analyzed: 18.61% of total proteins, 9.78% of crude fibre, 5.68% of ash, 25.48% of crude fat with iodine number -172.29, g J/100g of fat; acid number -2.07, mg KOH/g of fat and peroxide number -2.66, mmol H₂O₂/kg of fat.

Trial lasted 80 days, i.e. administration of the preparation started in dry period 20 days before calving and lasted 2 months during lactation. Both groups of cows received identical diets and trial group received in the diet flax preparation in the quantity of 0.7 kg in dry period and 1.2 kg during lactation. Diets for cows in dry period consisted of: meadow hay 8 kg, silage of entire maize plant 15kg, concentrate for dry cows (14%UP) – 4.5kg. Fresh cows were fed diets of following composition: alfalfa hay 6 kg, silage of entire maize plant 10 kg, sunflower meal 1,5 kg and concentrate mixture (18%UP)-5.0 kg. Cows in lactation were fed diet consisting of alfalfa hay – 5,0 kg; silage of entire maize plant - 25 kg, maize ear silage – 5.5kg, sunflower meal – 3.5 kg kg and 2 kg of concentrate mixture (18%TP) for production of 30 kg of milk. Nutrition parameters of the diet for lactating cows were following: dry matter – 20.6 kg; 140.00 MJ (NEL); 3210 g total protein and 4302.2 g of crude fibre.

In the laboratory of the Institute for Animal Husbandry, Belgrade-Zemun, chemical composition of livestock feeds and preparation “TradiLin” was established, content of macro and micro elements, as well as content of organic acids in the silage. Following methods were used: total protein - SRPS ISO 5983:2001; moisture SRPS ISO 6496:2001; crude fat SRPS ISO 6492:2001; crude fibre SRPS ISO 6865:2004; ash SRPS ISO 5984:2002; calcium in livestock feeds AAS IS-LDM-14; calcium in premix - IS-LDM-7; phosphorus in premix IS-LDM-5; total phosphorus in feeds -IS-LDM-4; micro elements- AAS IS-LDM-14; sodium - Rulebook^{A)} method 22; content of organic acids in the silage-IS-LDM-10; iodine number-SRPS ISO 3961:2001; peroxide number - SRPS ISO 3960:2001; acid number - SRPS ISO660:2000; level of acidity-volumetric method according by Soxhlet-Henkel.

Content of omega-6 and omega-3 fatty acids was done in the SP laboratory AD Bečej, using method MET 358 GC/MS SP.

Milk composition was measured by milkoscan apparatus. Somatic cell count was recorded according microscoping method (EN ISO 13366-1: 1997). The blood was withdrawn from jugular vein of lactating cows and biochemical parameters (glucose, protein, total bilirubin, aspartate aminotransferase-AST, alanine aminotransferase-ALT, total cholesterol, HDL and LDL cholesterol, calcium and phosphorus) were investigated from blood serum by automatic biochemical analyzer model Konelab 20.

The data from the experiment were developed statistically by using the computer program Statistica (Stat Soft Inc., Ver.6. 2003). Significance of mean differences was estimated by Student's t- test.

RESULTS AND DISCUSSION

Chemical composition of feeds and mineral content are presented in the Tab.1.

Tab. 1. Chemical composition of feeds

Parameter	Feeds						
	Alfalfa hay	Meadow hay	Maize silage	Maize ear silage	Concent rate (18%)	Sunflower meal	Premix
Total protein, %	15.01	6.61	2.07	5.25	18.88	36.44	-
Moisture, %	9.30	7.90	70.39	34.27	11.77	10.23	-
Crude fat, %	1.83	1.85	2.49	2.09	2.02	1.98	-
Crude fibre, %	32.96	33.02	6.77	4.90	7.60	15.45	-
Ash,%	7.11	10.65	1.47	1.00	6.36	6.78	-
Ca,%	1.20	0.29	1.5	0.28	1.02	2.92	-
P,%	0.29	0.42	0.6	1.43	0.73	9.95	-
NFE ⁶ ,%	33.79	39.97	16.81	52.49	53.37	29.12	-
Fe, mg/kg	-	-	-	-	-	-	1192
Cu mg/kg	-	-	-	-	-	-	1025
Mn, mg/kg	-	-	-	-	-	-	3555
Zn mg/kg	-	-	-	-	-	-	7348
Ca,%	-	-	-	-	-	-	20.32
P,%	-	-	-	-	-	-	3.84
Na,%	-	-	-	-	-	-	9.69

^{A)} Rulebook on methods of sampling and methods for physical, chemical and microbiological analysis of livestock feed (Official Journal SFRJ 15/87).

Tab.2. Content of organic acids and reviewing maize silage according by Flieg

	Content	Share%	Points	Assessment
Butyric acid	0	0	50	VERY GOOD
Acetic acid	0.8351	19.45	18	
Lactic acid	3.4577	80.55	98	
Total:	4.2928	100.00	98	

From presented results it is obvious that all quality parameters varied within limits characteristic for specific feed.

In Tab 3. the contents of omega-6 and omega-3 in milk were presented and it is noticeable that the content of omega-6 fatty acids in trial group compared to the control one increased by 0.13g/100 g of fat, which was statistically significant at the level of ($P \leq 0.05$), whereas the content of omega-3 fatty acids in the same order increased by 0.302g/100 g fat which resulted in statistical significance at the level of $P \leq 0.01$. Ratio between omega-6 and omega-3 in milk in the trial group was 5.360, and was much more favorable than in the control one which amounted to 10.602 .

Similar situation regarding the content of fatty acids in the dairy product called "mladi kajmak". So, content of omega-6 fatty acids in trial group had increased by 0.11 g/100 g of fats ($P \leq 0.05$), whereas the content of omega-3 had increased by 0.350g/100g of fats, expressed through statistical significance it was $P \leq 0.01$.

Tab.3. Content of omega fatty acids and their ratio in milk and »mladi kajmak«

Milk	Trial group	Control group
Omega-6 fatty acids, g/100g of fat	3.141 ^a ±0.1103	3.011 ^b ±0.024
Omega-3 fatty acids, g/100g of fat	0.586 ^A ±0.1632	0.284 ^B ±0.1933
Ratio omega-6/omega-3 fatty acids	5.360	10.602
“Mladi kajmak”		
Omega-6 fatty acids, g/100g of fat	3.90 ^a ±0.122	3.79 ^b ±0.061
Omega-3 fatty acids, g/100g of fat	0.76 ^A ±0.1123	0.41 ^B ±0.421
Ratio omega-6/omega-3	5.10	9.24

All value expressed as mean ± Sd

a,b-values in rows with different letters differ significantly ($P \leq 0.05$)

A, B-values in rows with different letters differ significantly ($P \leq 0.01$)

In Tab.4. durability of “mladi kajmak” is presented. Namely, “mladi kajmak” is declared as product with durability and taste which are maintained during 10 days in sale, and later the product undergoes fermentation and acquires characteristics of “stari kajmak”. “Mladi kajmak” is characteristic and popular product on the territory of Serbia. In this product, in refrigerator’s conditions the change in parameters of unsaturated fatty acids was monitored, i.e. inclination towards oxidation. Obtained results unambiguously indicate that “mladi kajmak” of the trial group enriched with omega-3 fatty acids, beside increased content of omega fatty acids has better durability compared to standard quality, since changes during the time occur slower, which was confirmed by lower values of peroxide number and level of acidity. In this way, nationally recognizable product which has been avoided by consumers due to health reasons and high content of saturated fats becomes foodstuff of high value with properties of functional food.

Tab.4. Durability of „mladi kajmak“

Parameter	Sampling period, days									
	Trial group					Control group				
	1	6	10	15	18	1	6	10	15	18
Iodine number, g J /100g fat	37.34					29.12				
Acid number, mg KOH/g fat	0.06					0.06				
Peroxide number, mmol H ₂ O ₂ /kg fat	1.08	1.14	1.44	1.46	1.58	1.12	1.23	1.54	1.56	4.88
Level of acidity, SH ^o	6.29	6.38	6.91	8.61	12.23	7.75	9.70	11.88	13.19	16.21

In Tab.5. chemical composition and quality of milk obtained from control and trial group are presented. Even though increased values of milk fat and proteins in trial group compared to control one were established, no statistical significance was exhibited because of high variations within groups. In both groups the somatic cell count, as well as total plate count, were at satisfactory level considering farm’s conditions i.e. it is result of good managing system.

Tab.5. The chemical composition and bacteriological quality of milk

	Parameter/Parametar					
	Milk fat, %	Protein, %	Lactose, %	Dry matter without fat, %	Somatic cell count in 1ml	Total plate count in 1 ml
T	4.01 ^a ±0.4359	3.72 ^a ±0.2646	4.42 ^a ±0.4899	8.82 ^a ±0.7141	175660 ^a ±233.48	<78000
C	3.80 ^a ±0.3000	3.39 ^a ±0.2449	4.74 ^a ±0.2646	8.94 ^a ±0.3464	208880 ^a ±326.46	<78000

*Values in rows with same letters not differ significantly $P > 0.05$

In the beginning and in the end of trial, the blood was taken from v. jugularis and the most important blood parameters were recorded. At the beginning of trial there were no differences in biochemical blood parameters, and at the end of trial period, as it can be seen from Tab. 6, all of them were in physiologically optimal limits (regardless of the fact that in

trial group levels of bilirubin and ALT, which resulted in statistical significance) which indicated stable health condition of heads of cattle and also that the stress occurring in this most productive physiological stage has been overcome. Physiologically optimal values were compared according by Kaneko (1989).

Tab. 6. Biochemical parameters of the blood

Parameter	Groups		Reference range
	Trial	Control	
Glucose, mmol/l	2.74±0.3606	2.91±0.2646	2.5-4.2
Total bilirubin, mmol/l	4.46 ^a ±2.2000	2.37 ^b ±1.1916	0.2-8.5
Aspartate aminotransferase (AST), U/L	112.95 ±36.2202	124±51.7409	78-132
Alanine aminotransferase(ALT), U/L	23.15 ^a ±9.2574	32.13 ^b ±9.2574	14-38
Total protein, g/l	77.19±2.7731	78.02±8.4202	67.4-74.6
Total cholesterol, mmol/l	3.58±0.7616	3.73±0.6245	1.6-6.5
HDL cholesterol, mmol/l	2.27±0.4796	2.25±0.4123	
LDL cholesterol, mmol/l	1.20±0.3873	1.39±0.2449	
Calcium, mmol/l	2.21±0.1414	2.21±0.2000	2.4-3.1
Phosphorus, mol/l	2.12±0.4690	2.15±8.4202	1.8-2.1

a, b-values in rows with different letters differ significantly ($P \leq 0.05$)

Feeding dairy cows a ration containing whole flax seeds (linseed), flax (linseed) oil or milled, extruded or micronized linseed has five beneficial effects on the fat profile of cow's milk: (1) increasing the content of alpha-linolenic acid (ALA); (2) increasing the content of conjugated linoleic acid (CLA); (3) decreasing the omega-6/omega-3 ratio; (4) decreasing the overall saturated fat content; and (5) increasing the proportion of stearic acid relative to other saturated fatty acids. These enhancements to the fat profile of milk give consumers value-added foods with good sensory qualities and a healthier fat profile (Morris, 2009).

When results obtained in this study are compared with results obtained by other authors it can be said that they are in correspondence with them. *Oba et al. (2009)*. obtained very important results by comparing the effects of α -linolenic acid in milk fat from Holstein cows which were fed diets supplemented by whole unprocessed, ground flax seed in the amount of 100g per kg of dry matter of diet. Problem occurs because of the specificity of digestion in ruminants where bio-dehydrogenation of unsaturated omega-3 fatty acids occurs, and in this way their digestibility is decreased unless they are made available, by processing procedure, to micro-organisms of rumen. Hypothesis that both feeding treatments influence the increase of concentration of α -linolenic acid in milk fat (8.3 and 8.6g/kg⁻¹) was confirmed and its concentration was three times higher in comparison to the one before the treatment, where instead of flax seed sunflower seed was used (2.6 g/kg⁻¹). Both treatments with whole unprocessed flax seed and ground seed indicate similar degree of absorption of α -linolenic acid in spite of lower digestibility of the whole unprocessed seed, which is noticeable by its content in faeces (259 vs. 129 g day⁻¹; $P < 0.001$). Ground flax seed doesn't increase significantly the absorption of α -linolenic acid in milk fat, since by increasing of its digestibility also the level of bio-dehydrogenation in rumen is increased.

Also, in research of *Petit (2002)* the effects of whole unprocessed flax seed as source of omega-3 fatty acids in diets for high yielding cows were studied and compared to other energy sources such as Megalac and micronized soy bean as energy source. It was concluded that inclusion of whole flax seed into diets for cows influenced not only the increase of quantity of milk (35.7kg/day compared to other two energy sources - 33.5 and 34.4kg/day), but also the protein content in milk as well as decrease of ratio of omega-6 and omega-3 fatty acids, which made it product of high quality from the aspect of human nutrition. Human diets rich in omega-6 fats result in a high omega-6/omega-3 ratio. A high dietary omega-6/omega-3 ratio is linked with low-grade chronic inflammation that contributes to diseases such as Alzheimer disease, cancer, coronary heart disease, metabolic syndrome, obesity, type 2 diabetes, osteoporosis and even dry eye syndrome (Morris, 2007) Reducing

the omega-6/omega-3 ratio helps decrease inflammatory reactions and lowers the risk of chronic disease. A dietary ratio between 4:1 and 10:1 is recommended (Gebauer, 2006). Improving the ratio can be achieved by eating less omega-6 fats, eating more omega-3 fats or doing both. Buying milk and meat products with a low omega-6/omega-3 ratio helps improve the dietary mix of fatty acids in the human diet (Morris, 2009).

CONCLUSION

Including the whole flaxseed (patented procedure of special flax cultivar) into dairy cows diet has the beneficial effects on the fat profile of cow's milk. This is a good and simply way of obtaining milk with functional food properties. Having in mind that milk enriched with omega-3 fatty acids has a favorable effects on human and animal health, inclusion of flaxseed through product called "TradiLin" has a full justification.

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