MILK FATTY ACID COMPOSITION AS A RESPONSE TO DIETARY N-3 FATTY ACIDS IN HOLSTEIN DAIRY COWS

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Abstract

Flax meal is known for its high content of alpha-linolenic acid, widely used as a source of fatty acid n-3 in animal feed. In this regard, a study on dairy cows was carried out to assess the influence of the use of flax meal on milk fatty acids. 15 dairy cows, Holstein breed (50 ± 23 days in lactation), with an average weight of 580 ± 37 kg, were divided into 3 experimental groups (C, E1, E2). Cows, were fed 2 times/day, and received a basic concentrate mix (corn, soybean meal, sunflower meal and wheat bran) and roughage (alfalfa hay and maize silo). Group C received the basic concentrate, while groups E1 and E2 received different formulations of concentrate by substituting sunflower meal (12%) for both groups (E1, E2). Besides, in the E2 group concentrate the wheat bran was substituted with barley rootlets (17.30%). Milk samples were collected in the morning and evening for a period of five consecutive days, on which the determinations of fatty acids were made using the chromatographic gas method. The use of flax meal in cows feeds resulted significant increase (P <0.05) in milk cows a of n-3 fatty acids at the end of the experiment for both groups fed flax meal, in the morning 0.53% and 0.54% in the evening (E1) 0.49% in the morning and 0.53% in the evening (E2) versus the C group (0.23%), representing a good source of omega 3. Such data indicated that the fatty acid addition was favourable to the fatty alpha-linolenic acid in milk.

Key words: n-3 fatty acids, milky cows, flax meal, barley rootlets

INTRODUCTION

Recent studies have attempted to determine in what proportion saturated fatty acid has been beneficial to human health. Haug et al. [1] determined the benefits of polyunsaturated fatty acids n-3 (PUFA) for human health. Since milk and dairy products are a substantial part of the human fat source, it would be beneficial to increase their n-3 fatty acid content [2]. In recent years, research has focused on the effect of racemates rich in polyunsaturated fatty acids n-3 (PUFA) on the milk fatty acid composition. Flax as a source of n-3 fatty acids has been widely used as it is well known for its abundant α-linolenic acid content [3]. It was reported [4] that the flax meal has a 68.57% level of linolenic acid. The addition of flax meal in the rations of dairy cows changes the composition of milk fat by lowering the ratio of saturated fatty acids and increasing the ratio of mono and polyunsaturated fatty acids. A three-fold increase in α-linolenic acid was observed in cows milk fed with an addition of flax seed [5]. The mechanism of saturation of bacterial fatty acids in rumen affects the growth of unsaturated fatty acids in milk [6]. It has been assumed that barley root will have an important role in feeding cows milk. The barley crude protein (CP) level is superior to maize and inferior to wheat, however it is considered to have the lowest quality and digestibility [7]. Cardova [8] in a dairy cow study, tested the substitution of maize with barley by-products and found that the average proportion of barley inclusion increased with neutral detergent fibre (NDF) and acid detergent fibres (ADF). The average dry matter consumption (DMI) decreased as the proportion of barley was increased. The ingestion levels for CP, NDF and ADF were

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not affected by the increased barley proportions in ration. In this context, the purpose of the research was to increase the ratio of α-linolenic acid in milk by replacing the sunflower meal with flax meal and to test the effect of replacing a portion of wheat bran with barley rootlets on the fatty acid concentration from milk and on haematological and biochemical parameters.

MATERIAL AND METHODS

The experiment was conducted on 15 multiparous Holstein dairy cows, body weight of approximately 580±37 kg, at 50±23 days of lactation. The cows were randomly allocated into three groups of 5 cows in separated pens, under same environmental conditions. In this experimental design a part of dietary n-6 fatty acids in control group (C) was replaced with n-3 fatty acids in two experimental flax meal and flax meal + barley rootlets (FM and FM+BR) groups keeping the diets iso-energetic and iso-nitrogenous. All cows were fed with the total mix ration which was offered to cows ad libitum and they were fed twice per day. The total mix ration was composed of corn silage 30 kg, alfalfa hay 6 kg and concentrate 8 kg per cow per day. Concentrate for the control (C) group contained corn, soybean meal, wheat bran, mineral premix and sunflower which was replaced with 12 % flax meal (FM) in the experimental 1 group (Table 1). In addition to that, in the experimental 2 (FM+BR) group, wheat bran (34.7%) from concentrate from group C, was replaced partially (17.30%) with barley rootlets (BR).

Table 1. Composition of the concentrate diets

Groups			
Ingredients, %	C	E1	E2
Corn	25	25	25
Bran	13	13	13
Soybean meal	12	12	12
Sunflower meal	12	0	0
Flax meal	0	12	12
Barley rootlets	0	0	17.30
Wheat bran	34.6	34.6	17.30
Calcium	1.10	1.10	1.10
Salt	1.30	1.30	1.30
Premix for cows	1.00	1.00	1.00

One experimental batch/group was manufactured; compound feed samples were collected and assayed for the basic chemical composition of the main nutrients. Standardized methods (according to CE Regulation 152/2009) were used to determine nutrients as follows: dry matter (DM), by the gravimetric method, drying at 103^0C, using Sartorius scales and BMT drying oven, ECOCELL Blueline Comfort; crude protein (CP), by Kjeldahl, method using the semiautomatic KJELTEC auto 2300 system – Tecator (Sweden); ether extractives (EE) by extraction in organic solvents, with SOXTEC-2055 FOSS system – Tecator (Sweden); crude fibre (CF) by the method with intermediary filtration, using FIBERTEC 2010 system – Tecator; ash (Ash) by the gravimetric method, using Caloris CL 1206 furnace; the minerals (calcium and phosphorus) were determined by inductively coupled plasma optical emission spectrometry, using Optima 5300 DV Perkin Elmer ICP-EOS spectrometer (Table 2). Milk and blood samples from each cow were collected on the 1st and last day of experiment. For the biochemical parameters we collected 1-2 mL venous blood, in vacutainer with not anticoagulant, with no separating el (green cap) for cholesterol, glycaemia and triglyceride assessment through spectrophotometry. We collected also 1–3 mL venous blood (depending on vacutainer volume), in vacutainers with EDTA (violet caps) and used ADVIA 2120i – Siemens: automat analyser of reference for veterinary haematology, based on flow cytometry, with peroxidase reaction and laser detection. The optical microscope was used to examine the blood smears. A sample/cow for five days in row of morning and evening milking on the 1st and last day of experiment was collected and mixed for fatty acids composition analyses. The fatty acids were determined by gas chromatography by transforming the fatty acids from the sample in methyl esters, followed by component separation in capillary column, identification by comparison with standard chromatograms and quantitative determination of the fatty acids according to SR CEN ISO/TS 17764 -2: 2008, using Perkin ElmerClarus 500 gas chromatograph, with capillary column injection system, high polarity stationary phase (BPX70: 60m x 0.25 mm inner diameter and 0.25µm thick film).

Experimental procedures were approved by the Ethical Committee of the National Research Development Institute for Biology and Animal Nutrition, in accordance with Romanian Law no. 305/2006 regarding handling and protection of animals used for experimental purposes. The analytical data were compared using variance analysis (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The experimental results were expressed as mean values ± standard deviation, the differences being considered statistically significant for $P < 0.05$.

RESULTS AND DISCUSSIONS

Because all treatments were analysed from one batch, it was not possible to statistically test differences in chemical composition among treatments. However, it was clear that all treatments (Table 2) were balanced iso-energetic and iso-nitrogenous.

Table 2 Chemical composition of compound feeds

Item	C	E1	E2
DM, %	88.03	88.07	88.33
OM, %	83.17	83.50	83.66
CP, %	20.08	20.10	20.20
EE, %	2.20	2.31	2.23
CF, %	7.37	7.31	7.45
Ash, %	4.92	4.74	4.74

DM-dry matter, OM-organic matter, CP-crude protein, EE-ether extractives, CF-crude fibre

From figure 1, regarding results for chemical composition of milk collected in the morning, it can be observed, that the highest level of CP it was found in the C group samples (3.16%). The EE content in E1 group was 4.31% compared with C group (4.12%) and E2 (4.21%). Although this difference is noticeably higher, but not statistically assured.

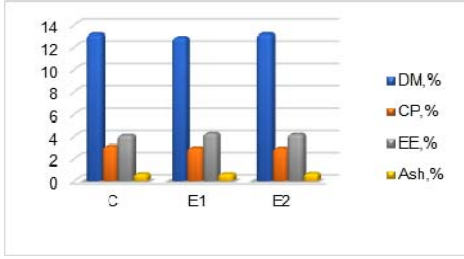

Figure 1. Chemical composition of milk collected in the morning

The results of analysed milk samples collected at evening milking, had the highest level of CP, and was determined in samples of group E2 (3.07%) compared to C group (3.05%). For milk fat, the E2 group registered the highest concentration (4.32%) compared to E1 (4.13%) and respectively (4.22%) in C group. These significant differences are not statistically assured, but many studies [9,10,11] had reported that the DM and CP are related with the milk yield.

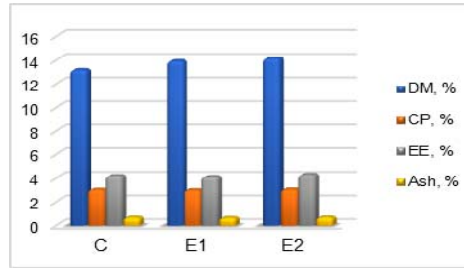

Figure 2. Chemical composition of milk collected in the evening

The proportions of milk fatty acids from samples collected on the 1st day of experiment, for morning and evening milks were similar as fatty acids composition. There were no differences between groups.

Feeding flax meal had an effect on milk fatty acids content (Table 3), in both experimental groups for both milking (morning and evening). The n-3 polyunsaturated fatty acids, were significantly higher ($P \leq 0.05$) in E1 (0.53% of total FAMEs) and 0,49% of total FAMEs in E2 compared with C group (0.24% of total FAMEs) for the first milk. In the second milk the E2 group was slightly higher (0.54% of total FAMEs) compared with E1 group (0.53% of total FAMEs) but both significantly higher than C group (0.23% of total FAMEs). The palmitic saturated fatty acid (C16:0), considered by the World Health Organization in the same group with the trans acids responsible for the higher risk of cardiovascular diseases [12], decreased in the milk fat of cows fed FM diet in both milks compared to FM+BR and C diet. Similar results were reported by [13]. According to [13], the fatty acids stored as triglycerides in ruminant adipose tissue are mainly C16:0, C18:0 and cis9 C18:1, and are dependent on nutritional history of the cow [14]. This can

be explained that the fatty acids determined in milk on last day of experiment came not only from dietary source but also from fat storages filled in previous lactation. This might be the explanation for lower PUFA and C 18:3n3 response in FM supplemented groups compared to the research of [15]. Also, for C20:3n6 and C20:3n3, respectively, significant differences ($P \leq 0.05$) were recorded between FM group and FM+BR respectively C group. Also, some authors compared milk composition after replacing saturated fats in feed with FM [16,17], and they also observed significant difference in n-3 PUFA from milk.

Table 3. Milk fat fatty acids composition (% of total FAMEs)

Fatty acids		C	E1	E2	C	E1	E2
		Morning samples			*Evening samples*		
SFA	C 14:0	12.81±1.4	13.47±0.5	13.87±0.9	13.35±1.23	14.13±0.7	14.01±0.9
	C 15:0	0.53±0.10	0.57±0.04	0.58±0.13	0.52±0.09	0.59±0.05	0.58±0.13
	C 16:0	36.07±2.8	33.94±3.8	36.46±3.2	36.50±2.9	34.53±4.1	36.56±3.3
	C17:0	0.45±0.07	0.48±0.04	0.47±0.12	0.48±0.05	0.46±0.03	0.51±0.10
	C 18:0	9.19±1.94	8.25±1.01	8.00±1.15	8.71±1.70	8.21±1.14	7.42±1.34
MUFA	C 14:1	1.34±0.32	1.46±0.16	1.49±0.26	1.40±0.22	1.53±0.22	1.51±0.32
	C 15:1	1.26±0.24	1.39±0.11	1.46±0.29	1.30±0.27	1.42±0.13	1.46±0.36
	C 16:1	2.47±0.43	2.53±0.30	2.16±0.25	2.38±0.44	2.26±0.18	2.33±0.21
	C 17:1	0.58±0.11	0.62±0.04	0.58±0.08	0.56±0.08	0.67±0.10	0.60±0.10
	C18:1n9c	20.10±3.2	20.80±2.2	18.57±1.7	19.24±2.1	19.55±1.9	17.74±1.5
n6-PUFA	C 18:3n6	0.07±0.04	0.08±0.03^{c}	0.03±0.02^{b}	0.06±0.03^{c}	0.09±0.01	0.10±0.01^{a}
	C20(2n6)	0.10±0.02	0.08±0.01	0.09±0.02	0.09±0.02	0.07±0.01	0.08±0.02
	C20(3n6)	0.09±0.04	0.12±0.02^{c}	0.07±0.01^{b}	0.06±0.02	0.10±0.03	0.09±0.03
	C20(4n6)	0.14±0.04	0.13±0.02	0.10±0.02	0.14±0.04	0.13±0.02	0.12±0.02
n3-PUFA	C 18:3n3	0.24±0.09^{bc}	0.53±0.07^{a}	0.49±0.14^{a}	0.23±0.09^{bc}	0.53±0.05^{a}	0.54±0.09^{a}
	C20(3n3)	0.08±0.02	0.10±0.00	0.08±0.02	0.08±0.02	0.08±0.02	0.09±0.02
∑SFA		69.34±4.18	68.14±2.8	71.20±2.6	70.21±3.85	69.62±2.4	71.34±2.3
∑UFA		29.47±4.02	30.85±2.7	27.80±2.6	28.63±3.7	29.32±2.3	27.62±2.2
∑MUFA		25.77±3.38	26.80±2.5	24.27±2.04	24.88±3.10	25.42±2.06	23.64±1.89
∑PUFA		3.70±0.88	4.05±0.30	3.53±0.61	3.76±0.90	3.90±0.29	3.98±0.37
Ω3		0.32±0.09^{bc}	0.62±0.07^{a}	0.57±0.16^{a}	0.31±0.11^{bc}	0.60±0.06^{a}	0.65±0.08^{a}
Ω6		3.05±0.78	3.11±0.25	2.69±0.42	3.14±0.78	2.97±0.24	3.01±0.31
Ω6/Ω3		9.77±0.60^{bc}	5.02±0.42^{a}	4.87±0.63^{a}	10.26±1.02^{bc}	4.95±0.35^{a}	4.63±0.22^{a}

Where: a,b,c significant differences ($P \leq 0.05$) compared to C, E1, E2. C - control group, E1 - experimental group with FM, E2 - experimental group with FM+BR; SFA - Saturated fatty acids, UFA-unsaturated fatty acids, MUFA - Monounsaturated fatty acids, PUFA - Polyunsaturated fatty acids.

It is well known, that diets containing relatively high proportions of polyunsaturated fatty acids, promote shifts in biohydrogenation pathways [18] and produce unique fatty acids intermediates which are potent inhibitors of milk fat synthesis [18].

The results in this study regarding the milk fatty acid composition of flax meal fed cows agree with the results of other authors [18]. The increase of PUFA and MUFA are similar with the results of other authors who fed dairy cows with flax meal [19]. In the present study the increase of PUFA ratio in milk was not as consistent as some other authors reported by using extruded flax meal [14] or micronized flax meal [20]. Also, some authors studied the milk composition after replacing saturated fats in feed with flax meal [15,12]. In this study we have determined an increase ($P<0.05$) of α-linolenic acid in milk of both groups compared to the C group. Also, the essential fatty acids for human health, were higher in experimental groups, compared with C group.

Blood plasma parameters had almost the same value. The differences between means of blood plasma parameters, were not significant as presented in Table 4. The biochemical parameters determined in the serum (Table 4) revealed several benefits of feeding the flax meal to dairy cows. The glycaemia parameter of the energy plasma profile was significantly ($P \leq 0.05$) lower in experimental groups compared with C group. The cholesterol and triglycerides were higher

in experimental groups feed FM and FM+RB compared to group C, but we cannot explain what was the reason. We can assume that the diets rich in fatty acids, has some influences on blood plasma. Some studies [21] also show no significant differences in cows blood samples. The same trend was noticed in the mineral and enzymatic profile. The values determined for the haematological parameters, which show the health state of the cows (Table 4) ranged within the normal values reported in the literature [22, 21].

Table 4. Biochemical and haematological parameters (average values/group)

Specification	C	E1	E2
Serum biochemical parameters*			
Energy plasma profile			
Glycaemia, (mg/dl)	45.01±4.6	34.00±4.92	25.57±2.90
Cholesterol, (mg/dl)	127.57±24.234	140.00±21.01	142.76±23.32
Triglycerides, (mg/dl)	2.93±2.7	2.44±2.87	3.28±3.52
Protein profile			
Albumin, (mg/dl)	2.6±0.548	2.6±0.548	2.6±0.54
Total bilirubin, (mg/dl)	0.1±0.02	0.07±0.01	0.08±0.01
Total protein, (g/dl)	7.70±1.23	5.90±0.33	5.83±0.74
Creatinine, (mg/dl)	0.85±0.11	0.89±0.13	0.94±0.07
Urea, (mg/dl)	15.29±6.80	11.90±2.68	16.32±2.42
Mineral profile			
Calcium, (mg/dl)	8.06±0.95	7.25±0.71	7.23±1.08
Phosphorus, (mg/dl))	4.84±1.02	4.47±0.40	5.77±0.79
Magnesium, (mg/dl	2.13±0.41	1.84±0.21	1.83±0.15
Iron, (ug/dl)	128.15±31.70	114.64±21.13	102.18±23.49
Enzyme profile			
Alt (TGP), U/L	28.76±9.14	27.93±4.51	27.96±4.49
Ast (TGO), U/L	84.40±25.95	63.08±5.91	86.45±23.26
Alkaline phosphatase, U/L	40.41±20.39	35.03±7.99	35.69±7.81
Gama GT,	28.68±5.29	19.59±1.85	29.58±3.64
Haematological parameters **			
Leucocyte m/mm	16.54±14.23	9.14±2.70	8.11±1.63
Lymphocyte, %	45.62±25.90	39.7±13.30	38.36±4.86
Monocyte, %	5.12±2.52	5.96±1.07	6.6±1.11
Neutrophil, %	24.52±11.32	30.22±7.64	33.44±4.03
Eosinophil, %	24.22±13.17	23.62±5.72	21.3±5.01
Basophile, %	0.52±0.32	0.5±0.2	0.3±0.15
Erythrocyte, m/mm	5.41±0.67	5.41±0.45	5.52±0.34
VEM, fl	44.12±1.68	46.04±2.49	44.06±1.00
Hct, %	23.88±3.11	24.88±2.56	24.34±1.92
Hem, pg	16.30±0.26	16.22±0.59	16.18±0.30
Chem, g/dl	37.06±1.35	35.44±1.27	36.78±0.98
IDE	11.66±0.84	12.06±0.99	11.44±0.56
RRg	2.68±2.02	5.87±3.65	5.97±4.62
Hb, g/dl	8.86±1.19	8.82±0.86	8.96±0.68
PLT, m/mm	191.4±44.69	172.4±19.69	189.4±81.81
VPM, fl	10.60±0.45	10.60±0.55	10.70±0.50
Pct, %	0.20±0,05	0.18±0.03	0.20±0.09
IDP	7.40±1,40	7.84±0.54	7.85±0.62
PLCR, %	8.50±3.70	5.40±2.70	6.40±4.39

Where a,b,c, = significant differences (P≤0.05) compared to C, E1, E2. *(Merk, 2014) **reference values according to: Weiss D.J., Wardrop K.J. - Schalm's Veterinary Hematology, 6th Ed., 2010, Ed. Blackwell, pp. 965 and Jain, 1993.

CONCLUSIONS

Despite the limited available published data, some rigorous researches are needed on the influence of flax meal on blood parameters and fatty acids composition of milk. Feeding dairy cows with flax meal acted positively on milk fatty acid composition by increasing the ratio of n-3 fatty acids and the omega3. When supplementing flax meal in a practical range of feeding fat to dairy cows, the milk fatty acid

profile was improved with increased potentially human health-beneficial fatty acids.

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REFERENCES

[1] Haug, A., Hostmark, A.T., Harstad, O.M. 2007: Bovine milk in human nutrition - a review, Lipids in Health and Disease, 6 (25), 1-16. https://doi.org/10.1186/1476-511X-6-25.

[2] Gantner, V., Mijić, P., Baban, M., Škrtić, Z., Turalija, A. 2015: The overall and fat composition of milk of various species. Mljekarstvo 65 (4), 223-231.

[3] Scholljegerdes, E.J., Lekatz, L.A., Vonnahme, K.A. 2014: Effects of short-term oilseed supplementation on plasma fatty acid composition, progesterone and prostaglandin F metabolite in lactating beef cows. Animal 8, 777-785.

[4] Vlaicu, P. A., Panaite, T. D., Voicu, I., Turcu, R. P., Olteanu, M., & Ropota, M. 2018: Determining the feeding value of some food industry by-products. Scientific Papers: Animal Science and Biotechnologies, 51(1), 62-69.

[5] Moallem, U. 2009: The effects of extruded flaxseed supplementation to high-yielding dairy cows on milk production and milk fatty acid composition. Animal Feed Science and Technology 152, 232-242.

[6] Lourenço, M., Ramos-Morales, E., Wallace, R.J. (2010): The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal, 4, 1008-1023. https://doi.org/10.1017/S175173111000042X

[7] Zardo, A. O.; Lima, G.J.M.M. 1999: Alimentos para Suínos. Boletim Informativo de Pesquisa - Embrapa Suínos e Aves e Extensão - EMATER – RS, nº12, ano 8.

[8] Cardova, H. A., 2004: Utilização de cevada em substituição ao milho em dietas para vacas holandesas de alta produção. [Thesis Magoster]. Programa de Pós graduação em Ciências Agroveterinárias: Universidade do Estado de Santa Catarina.99p

[9] Doreau, M., and A. Ferlay. 2015. Linseed: A valuable feedstuff for ruminants. OCL 22:D611.https://doi.org/10.1051/ocl/2015042

[10] Oeffner, S. P., Y. Qu, J. Just, N. Quezada, E. Ramsing, M. Keller, G. Cherian, L. Goddick, and G. Bobe. 2013. Effect of flaxseed supplementation rate and processing on the production, fatty acid profile, and texture of milk, butter, and cheese. J. Dairy Sci. 96:1177–1188.

[11] Rabiee, A. R., K. Breinhild, W. Scott, H. M. Golder, E. Block, and I. J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and metaregression. J. Dairy Sci. 95:3225–3247.

[12] Petit, H.V., Palin, M.F., Doepel, L. (2007): Hepatic Lipid Metabolism in Transition Dairy Cows Fed Flaxseed. Journal of Dairy Science 90, 4780-4792. https://doi.org/10.3168/jds.2007-0066

[13] Gonthier, C., Mustafa, A.F., Ouellet, D.R., Chouinard, P.Y., Berthiaume, R., Petit, H. V. (2005): Feeding micronized and extruded flaxseed to dairy cows: Effects on blood parameters and milk fatty acid composition. Journal of Dairy Science, 88, 748-756.

[14] Bauman D E and Griinari J M. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition 23: 203–27.

Zened A, Enjalbert F, Nicot M C and Troegeler-Meynadier A. 2013. Starch plus sunflower oil addition to the diet of dry dairy cows results in a trans-11 to trans-10 shift of biohydrogenation. Journal of Dairy Science 96: 451–59.

[15] Voicu D, Vasilachi A and Voicu I. 2017. Influence of feeding dried grape marc to fattening steers on the fatty acids composition of the hepatic tissue. Indian Journal of Animal Sciences 87(9): 1141–44.

[16] AbuGhazaleh A A, Schingoethe D J, Hippen A R and Kalscheur K F. 2004. Conjugated linoleic acid increases in milk when cows fed fish meal and extruded soybeans for an extended period of time. Journal of Dairy Science 87: 1758–66.

[17] Mislav Đidara, et al., 2017: Plasma and milk fatty acid composition as a response to dietary n-3 fatty acids and selenium in periparturient Holstein cows. 67 (2), 123-129.

[18] Chilliard, Y., Ferlay, A., Mansbridge, M.R., Doreu, M. (2000): Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Annales De Zootechnie 49, 181-205. https://doi.org/10.1051/animres:2000117.

[19] Marenjak, T.S., Delaš, I., Poljičak-Milas, N. (2009): The milk fatty acid composition and production in Simmental cows supplemented with unprotected sunflower oil in the ration. Milchwissenschaft 64 (2009), 3; 235-238.

[20] Brscic, M., Cozzi, G., Lora, I., Stefani, A.L., Contiero, B., Ravarotto, L., Gottardo, F. Short communication: reference limits for blood analytes in Holstein late-pregnant heifers and dry cows: effects of parity, days relative to calving, and season. J. Dairy Sci. 2015;98:7886–7892.

[21] Weiss, D. J., Wardrop, K. J., Schalm's Veterinary Hematology, 6th Ed., 2010, Ed. Blackwell, pp.965.

[22] Jain, N. C., New York Univ., NY (USA). School of Medicine. Depts. of Medicine and Microbiology. Essentials of veterinary hematology, 199).