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Milk yield, physico-chemical parameters and fatty acid content from dairy cows fed two types of non-genetically modified soybean cakes

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ABSTRACT

This study evaluated the inclusion of two types of cakes obtained from non-genetically modified soybean cultivars (cv. Regale, Italy and cv. Onix, Romania; RSBC and OSBC diet, respectively) in dairy cows compound feeds in order to assess their effects on the quantity (yield) and quality (physico-chemical parameters and fatty acids composition) of raw milk.The feeding trial used 16 multiparous Romanian Black Spotted dairy cows, mid-lactation stage, with an initial average milk yield of 18.46 litres/d, assigned to two groups (8 cows each) for 43 days. Milk yield and milk protein were not influenced by the feeding of RSBC or OSBC diet. However, the OSBC diet decreased significantly milk fat content (3.71% vs. 4.08%). Palmitic acid content in milk fat of dairy cows receiving the RSBC diet was significantly lower in comparison with the OSBC diet (30.27% vs. 32.03%). The conjugated linoleic acid content was significantly higher with the RSBC diet, while the total polyunsaturated fatty acids content did not differ among treatments.

Key words: Dairy cows, Fatty acids, Milk, Non-GMO, Soybean cake

Soybean (*Glycine max*) provides superior quantitative and qualitative nutrients for human and animal consumption, as well as a number of by-products for manufacturing. Among ruminant feedstuffs, the use of soybean by-products (e.g. soybean meal) ranks first place due to some features, viz. high level of ruminal degradable protein (over 60%), balanced amino acid content, good digestibility of the cell wall and high palatability (INRA 2007). However, this by-product comes mostly from genetically modified crops and this situation encourages European Union countries to find ways to reduce the dependence on imported protein feeds (Dima 2015, Ciurescu *et al*. 2017). In this regard, in Romania, the soybean breeding program had as priority the creation of early productive varieties, non-genetically modified, but ameliorated for a high yield potential, high protein and oil content, good level of resistance to pathogens and suitable for mechanized harvesting (Muresanu *et al.* 2003, ISTIS 2016). On the other hand, as the consumer perceptions of food has changed, the challenge in ruminant nutrition is to enhance nutritional benefits of end products, like decreased milk fat content or increased polyunsaturated fatty acids concentration (Lock and Bauman 2004, Jenkins and McGuire 2006) and to provide functional foods for humans, obtained from healthy animals fed with feed free of any genetically modified constituents. Lately, in Romania, the considerable increase of soybean production and improvement of the processing methods have led to the achievement of high nutritional value by-products and the supply of protein which allows increased quantities of animal products (Gãgeanu 2012). For example, cold pressing of oilseeds has the advantage that in addition to the production of vegetable oil, valuable feed cakes are also obtained and may be successfully used in animal husbandry. Therefore, soybean cakes (SBC) may be a low cost by-product of oil processing which can be used as both protein and lipid sources for livestock.

In this context, the purpose of the study was to test two types of SBC obtained from non-genetically modified soybean cultivars (cv.), in order to determine their effects on the quantity (yield) and quality (physico-chemical parameters and fatty acids composition) of raw milk.

MATERIALS AND METHODS

All 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.

Animals and diets: Sixteen multiparous [parity number 2.81 (SD=0.75)] Romanian Black Spotted dairy cows with 610.73 kg average body weight (SD=55.67), and an initial daily milk yield of 18.46 litres/d (SD=1.52) and 179 DIM (SD=76.5) were used in a monofactorial design for 43 days

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experiment.The animals were randomly assigned to 2 homogeneous groups of 8 cows each and received the same basal diet: spring hay (60% oat hay + 40% vetch hay) and alfalfa haylage. Dietary treatments consisted of two compound feeds (CFs) differentiated by the proteic ingredient. Thus, two types of SBCs obtained from non-genetically modified soybean seeds (cv. Regale, Italy and cv. Onix, Romania; RSBC and OSBC diet, respectively) were used. The two sources of SBCs were manufactured by cold pressing procedure and included 14% of CFs. The energetic ingredients for both CFs were corn (35.7%), barley (17.0%) and wheat bran (30%).The CFs were completed with 1.1% $CaCO_3$, 1.2% salt and 1.0% vitamin-mineral premix. To ensure optimal growth of microorganisms in the rumen, the ratio between forages and CFs was 65.8:34.2. Diets (basal plus CFs addition) were formulated in line with nutritional requirements adapted to live weight, milk production, fat percentage and physiological status (Burlacu *et al.* 1991, 2002), were isoenergetic and isonitrogenous and had the same nutritional characteristics (19.0 DM kg/cow/d, 18.0 milk feed units, FU_{milk}/cow/d and 1440 g intestinally digestible protein, IDP/cow/d). Feed was offered twice daily and the access to water was permanent (constant level drinkers).

Data collection, sampling, analysis and statistics: Amounts fed, refusals and milk yield were recorded daily. Feed samples were analysed for the fatty acid (FA) content. Milk samples (3 series of samples collected from each animal from 2 to 6 weeks resulting a total of 24 samples/diet) were analysed for physico-chemical parameters [fat, solids non fat (SNF), protein, lactose, acidity, conductivity and pH] using an ultrasonic milk analyzer (EkoMilk Bond Total) and for the FAs content with a Perkin Elmer Clarus 500 GC.The results were expressed for each FAs as % of total Fatty Acid Methyl Esters (FAMEs). All the data were statistically processed using General Linear Model (GLM) procedure of IBM SPSS Statistics (version 20, 2011). One-way analysis of variance (ANOVA) was used to check the differences between the results obtained from the two groups of animals at 0.05 level of significance. For P between 0.05 and 0.10 level of significance, it was considered that the factor of influence (diet) tended to influence the animals response. The results are given as means with standard error of means (SEM).

RESULTS AND DISCUSSION

The FAs composition of the feeds used in this experiment is shown in Table 1. The two types of SBC had similar nutritional characteristics. Both sources were characterized by a low content of saturated fatty acids (SFA, from 12.92 to 13.67%) and a high content of unsaturated fatty acids (UFA, from 86.34 to 87.07%) of which over 60% were mainly polyunsaturated fatty acids (PUFA). Compared to Regale, Onix soybean cakes contained 1.10 times more n-3 FAs resulting in a slightly lower n-6/n-3 ratio (by 9.0%). These results were in accordance with the literature databases (Nita *et al.* 2015, Heuzé *et al.* 2017). The CFs had also comparable FAs concentrations but the RSBC diet contained less n-6 FAs (by 6.0%) compared to OSBC diet.

Table 1. Fatty acids composition of soybean cakes and compound feeds (% of total FAMEs)

Item	Regale soybean cake	Onixsoy bean cake	Compound feed	
			RSBC	OSBC
C16:0	9.86	9.02	16.62	17.28
C18:0	3.74	3.40	3.21	2.85
C18:1n9	23.20	22.79	27.04	24.36
C18:2n6	54.21	51.96	46.53	49.57
C18:3n3	7.83	9.49	3.75	3.81
Others	1.16	3.34	2.85	2.13
Σ SFA	13.67	12.92	20.57	20.86
Σ UFA	86.34	87.07	79.15	79.14
Σ MUFA	23.28	22.88	28.12	25.17
Σ PUFA	63.06	64.19	51.03	53.97
Σ n-3	8.85	9.74	4.35	4.29
Σ n-6	54.21	54.45	46.68	49.68
n-6/n-3	6.13	5.59	10.73	11.58

Table 2. Milk yield and physico-chemical parameters

Item	Diet		SEM	P-value
	RSBC	OSBC		
Milk yield (litres/d)				
Initial	18.54	18.38	0.56	0.839
Final	19.45	19.28	0.17	0.535
Physico-chemical parameters				
Fat (%)	4.08^{a}	3.71^{b}	0.10	0.014
SNF (%)	8.35	8.26	0.08	0.429
Protein (%)	3.17	3.13	0.03	0.408
Lactose (%)	4.56	4.51	0.05	0.466
Acidity (°T)	19.69	20.05	0.20	0.219
Conductivity (mS/cm)	4.47^{b}	4.72^{a}	0.07	0.010
pH	6.51	6.49	0.01	0.269

Values bearing different superscripts within the same row are different.

Milk-yield and physico-chemical parameters are shown in Table 2. No effect of treatments was observed on final milk yield (P=0.535), but the milk production recorded a plus compared with the initial phase of the experiment. The equal production with the two diets suggests that proteins had similar extents of ruminal escape, although small response of 0.9 l of milk can also imply that the specific esential amino acids may have been limited. It is possible that the cold pressing treatment to not maximize the amount of available lysine passing to the small intestine. Among milk physico-chemical parameters, no differences (P>0.05) was observed for SNF, protein, lactose, acidity and pH, but the milk fat percentage was significantly lower (P=0.014) and the conductivity was significantly higher (P=0.010) in the group fed OSBC diet. However, fat is known to be the most variable parameter of milk and may be easily influenced by nutrition (Jenkins and McGuire 2006), non-nutritional factors (Lanier and Corl 2015), intense genetic selection for milk yield (Kay *et al*. 2005) or may be partial explained by the fact that cows may have inherently low milk fat content especially when feeding similar diets. Likewise, diets containing relatively high proportions of PUFA are known to promote shifts in biohydrogenation pathways (Bauman and Griinari 2003) and to produce unique FAs intermediates which are potent inhibitors of milk fat synthesis (Zened *et al.* 2013). Also the relative sensitivity of mammary lipogenic processes is involved in the synthesis of milk fat (Shingfield *et al.* 2010). Other studies reported that decreases in milk fat output may be associated with an increase in milk oleic trans (C18:1) concentrations (Bauman and Griinari 2001), especially trans-10 C18:1 content (Piperova *et al.* 2000), and trans-10 cis-12 conjugated linoleic acid (CLA) (Baumgard *et al.* 2000, Lock *et al.* 2006). Propionate as well tends to decrease milk fat synthesis in lactating cows (Rulquin *et al.* 2007). Previous investigations showed that the use of dietary processed oilseeds (soybean, sunflower and linseed cakes) can affect the composition of milk (Horký 2014). However, our results regarding the values of all physico-chemical parameters of milk were within the breed ranges.

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

Item	Diet		SEM	P-value
	RSBC	OSBC		
C4:0	0.13	0.12	0.02	0.727
C6:0	1.43	1.48	0.04	0.461
C8:0	1.40	1.39	0.03	0.952
C10:0	3.11	3.04	0.10	0.608
C11:0	0.36	0.36	0.01	0.864
C12:0	3.47	3.47	0.11	0.992
C13:0	0.09	0.10	0.01	0.389
C14:0	12.48	12.50	0.22	0.934
C14:1	1.06	1.01	0.04	0.403
C15:0	0.56[b]	0.61[a]	0.02	0.033
C15:1	1.20[b]	1.31[a]	0.02	0.003
C16:0	30.27[b]	32.03[a]	0.44	0.007
C16:1	1.50	1.60	0.05	0.159
C17:0	0.48[b]	0.52[a]	0.01	0.011
C17:1	0.61[b]	0.66[a]	0.01	0.001
C18:0	10.41	9.89	0.26	0.162
C18:1n9t	0.09	0.13	0.06	0.615
C18:1n9c	24.09	22.74	0.49	0.057[T]
C18:2n6t	0.73	0.68	0.03	0.305
C18:2n6c	3.08	2.91	0.14	0.402
C18:3n6	0.05	0.07	0.01	0.190
C18:3n3	0.70	0.70	0.02	0.861
CLA	0.49[a]	0.41[b]	0.02	0.001
C20:0	0.04	0.06	0.01	0.086[T]
C20:2n6	0.10	0.12	0.02	0.442
C20:3n6	0.09	0.11	0.02	0.421
C20:3n3	0.06	0.08	0.02	0.500
C20:4n6	0.15	0.17	0.03	0.679
ΣSFA	64.22	65.57	0.61	0.125
ΣUFA	34.00	32.69	0.61	0.138
ΣMUFA	28.54	27.45	0.48	0.114
ΣPUFA	5.46	5.24	0.18	0.415

Different superscripts within the same row are different

Feeding SBCs had an effect on milk FAs content (Table 3), and the effect was sightly marked with the RSBC diet. Even though diets include a high proportion of UFAs, ruminant meat and milk contain much higher levels of SFAs due in part, to extensive biohydrogenation of dietary UFAs in the rumen (Shingfield *et al.* 2010). Also, FAs *de novo* synthesis accounts for all butyric (C4:0) to lauric (C12:0), most of the myristic (C14:0;~95%) and about 50% of palmitic (C16:0) acids are secreted in milk, whereas all C18 carbon and longer chain FAs are thought to be derived from circulating plasma lipids (Chilliard *et al.* 2000). Although not significant, the RSBC diet increased the proportion of UFAs. This effect was mainly due to an increase in monounsaturated fatty acids (MUFA) content, especially C18:1n9c, who had an tendency to be influenced by the diet (P=0.057). On the other hand, the lower values of stearic (C18:0) and C18:1n9c acids from the milk fat of cows fed OSBC diet (9.89 vs. 10.41 and 22.74 vs. 24.09, respectively) represents a positive aspect from nutritional point of view. The RSBC diet led to lower levels of medium-chain MUFAs, particularly of pentadecenoic (C15:1) and heptadecenoic (C17:1) acids (P<0.05), while myristoleic (C14:1), palmitoleic (C16:1) and C18:1n9t had comparable values. The C16:0, considered by the World Health Organization in the same group with the trans acids responsible for the higher risk of cardiovascular diseases (Voicu *et al.* 2017), decreased significantly (by 1.06 times) in the milk fat of cows fed RSBC diet compared to OSBC diet, as well as pentadecanoic (C15:0) and heptadecanoic (C17:0) respectively. This fact should be taken into consideration especially because the C16:0 together with C12:0 and C14:0 are known as reputed atherogenic acids. The OSBC diet compared to RSBC diet decreased the content of trans-6 and cis-6 linoleic acid (C18:2) with 7% and 6% respectively. Despite the diets content of alpha-linolenic acid (ALA, C18:3n3), no differences were observed in milk concentration of this FA, therefore, SBCs compared with other oilseeds plant (e.g. flax) may be less efficient in increasing milk ALA content but this is also very likely to be related with a substantial hydrogenation in the rumen. However, these results were satisfactory because the milk fat n-6: n-3 ratio (5.30 vs. 5.52) was slightly lower (by 4%) in the cows fed OSBC diet (AFSSA 2003 recommendations are ≥ 5). The RSBC diet increased significantly the milk content of total CLA, by 1.20 times, and tended to decrease the arachidic acid (C20:0). These results were similar with AbuGhazaleh *et al.* (2004). As for eicosadienoic (C20:2n6) and eicosatrienoic acids (C20:3n6 and C20:3n3, respectively), no significant differences were observed.

The two types of SBCs, obtained from non-genetically modified soybean cultivars, are equivalent for use in dairy cows feeds. Thereby, ameliotared protein sources may enhance animals end products closer to human demands. Although significant amounts of PUFAs are given to dairy cows, the outflows into the milk fat are limited to about 10% or less.

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