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Effect of dietary supplementation with *Saponaria officinalis* root on rumen and milk fatty acid proportion in dairy cattle*

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The objective of the present study was to evaluate the impact of powdered *Saponaria officinalis* (SO) root on fatty acid (FA) proportion in the rumen fluid and milk of dairy cows. The SO is a good source of triterpenoid saponins and it can be considered as a promising, natural additive for modulating FA proportion in ruminant's milk. Three rumen cannulated Polish Holstein cows were assigned to a 3 x 3 Latin square design. The cows received the following treatments: basal diet (Control), basal diet supplemented with either 440 g/day/animal (SO1) or 660 g/day/animal (SO2) of *S. officinalis* root. In the rumen fluid of cows fed SO2, *trans*-11 C18:1, *cis*-9, *cis*-12 C18:2 and total *n*-6 FA were significantly elevated, while concentration of *cis*-9, *trans*-11 C18:2 decreased. The SO2 diet significantly increased the milk yield and milk fat content. As far as the milk is concerned, the proportion of five FAs increased in response to SO treatment (*trans*-11 and *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2 and its conjugated isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12). The results demonstrated that powdered SO root may effectively modulate milk FA proportion in dairy cows.

KEYWORDS: **dairy cows / fatty acid / rumen and milk / saponins**

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It is well documented that the proportion of unsaturated fatty acids (UFA) in ruminant products can be increased by dietary supplementation with plant bioactive compounds and/or oils rich in linoleic and linolenic acid [Jóźwik *et al.* 2016]. Oils affect microorganisms in terms of synthesis of the conjugated linoleic acid (CLA) precursors whereas plant secondary metabolites can alter the activity of rumen microorganisms and UFA biohydrogenation (BH) [Szczechowiak *et al*. 2016, Zymon *et al*. 2014]. Generally, dietary supplementation with biologically active compounds of plant origin reduces formation of the saturated fatty acids (SFA) and elevates UFA concentration (mainly CLA) in the rumen and in ruminant products (milk and beef) [Jóźwik *et al*. 2010] and Strzałkowska *et al*. [2009]. Thus, the potential advantage of such products for human health is enhanced [Koba and Yanagita 2013]. Among many bioactive compounds of plant origin, saponins seem to be an appropriate choice [Szczechowiak *et al.* 2013]. *Saponaria officinalis* (SO) is a source of triterpenoid rich saponins [Cieslak *et al.* 2014]. The hitherto published reports on the saponin impact on rumen fatty acid (FA) proportion is scarce and mostly concern *in vitro* conditions. Conclusions based on *in vitro* derived data should be however treated with caution, and *in vivo* research is indispensable [Benchaar and Chouinard 2009]. To date, limited information on possible effect of saponins on the FA metabolism in ruminants has been published [Brogna *et al*. 2011].

Hence, the objective of the present study was to investigate the impact of two dosages of powdered SO roots as the source of saponins, on milk yield and milk composition, as well as on the FA proportion in the rumen fluid and milk of Polish Holstein Friesian cows.

Material and methods

Experimental design, dairy cows, and treatments

The experiment was conducted on three lactating Polish Holstein Friesian cows fitted with a rumen cannulas. The animals used in the study were in their 2nd lactation, and began the experiment on 120th day of lactation. The cows were assigned to a 3 x 3 Latin square design and randomly assigned to one of the three dietary treatments, at the 1st cycle. Each of the three experimental cycles lasted 26 days, including a 23-day adaptation period (adapting the animals and their rumen microflora to the experimental diets), and a 3-day sample collection period. The control diet was balanced and formulated according to the INRA standards to contain 1595 units of truly digestible protein in the small intestine and 16.0 units for milk yield [INRA 1993]. The diets were prepared daily. The control diet consisted of a total mixed ration (TMR) based on corn silages (7.4 kg dry matter; DM), ensiled brewer's grains (1.2 kg DM), ensiled beet pulp (0.4 kg DM), meadow hay (1.3 kg DM), rapeseed meal (1.6 kg DM), and a commercial concentrate (6.1 kg DM; 19% of crude protein). The experimental diets were based on the control diet supplemented with either 440 g/d (SO1) or 660 g/d (SO2) of powdered SO root. The SO dosages were defined based on the results of

previous *in vitro* [Szczechowiak *et al.* 2013, Cieslak *et al*. 2014] and *in vivo* studies [Szkudelska *et al*. 2016]. We have previously shown that supplementing ruminant diets with SO can modulate *in vitro* rumen fermentation and does not negatively influence the blood parameters in dairy cows [Szkudelska *et al*. 2016]. The cows were fed twice a day (05:00 and 17:00) in individual box feeders with free access to water and mineral saltlick. During the first 8 days of the adaptation period, the cows were fed *ad libitum* and powdered SO root was introduced gradually to avoid negative impact on DM intake. From day 9 onwards, the feed was restricted to 95% of the average daily voluntary DM intake basing on individual data recorded from day 3 to day 8 of the adaptation period, to avoid confounding effects of DM intake. Cows were milked daily at 05:00 and 17:00 and the milk yield was recorded.

Sampling and chemical analysis

Representative samples of the TMR and powdered SO root were collected on each day during sample collection period. The samples were stored at -20°C until analysis. Samples of the TMR and powdered SO root were analysed according to the AOAC [2007] for DM (method no. 934.01) and ash (method no. 942.05). Crude protein was determined using a Kjel-Foss Automatic 16210 analyser (method no. 976.05). Crude fat was determined using the Soxtec System HT analyser (method no. 973.18). Neutral detergent fiber (aNDF; estimated with amylase and sodium sulphite and expressed without residual ash), were determined by the method of Van Soest *et*

Table 1. Chemical composition and fatty acid proportions (g/100 g of total fatty acids) of the total mixed ration (TMR) and *Saponaria officinalis* powdered root (SO)

Item	TMR	SO
Basic components (g kg^{-1} of DM)		
Organic Matter	894	917
Ash	86	83
Crude Protein	171	104
Crude Fat	43	5
Neutral-detergent fiber	425	206
Fatty acid proportions (g/100 g of total fatty acids)		
C12:0	0.53	not detected
C14:0	0.82	not detected
C16:0	22.20	28.81
C18:0	3.76	4.29
cis-9 C18:1	18.50	14.91
cis-9, *cis*-12 C18:2	42.87	28.90
cis-9, *cis*-12, *cis*-15 C18:3	7.15	3.51
SFA	28.41	33.47
UFA	71.59	66.53
MUFA	21.35	32.40
PUFA	50.24	34.13
n-6	43.10	29.62
n-3	7.15	4.52

al. [1991]. Organic matter was calculated as the difference between DM and the ash contents. The chemical composition and FA proportion of TMR and powdered SO are presented in Table 1.

The concentrations of bioactive compounds in powdered SO root were analysed according to the protocol described by Cieslak *et al*. [2014]. The identified bioactive compound fractions were: polysaccharides (310 g/kg DM), saponins (91 g/kg DM), and polyphenols (17 g/kg DM).

During the sampling period, the rumen content (500 ml) was taken from the front ventral, middle ventral and cranial dorsal sac of the rumen through the cannula, directly before the morning feeding, and then 3 and 6 hours after the morning feeding. Immediately after withdrawal, the rumen content was strained through four-layer cheesecloth to separate the rumen fluid. Milk samples taken in the morning and evening were analysed separately. The basic milk constituents were analysed directly after milking (Milko-Scan 255 A/S N, Foss Electric, Hillerod, Denmark). Samples for FA detection were stored at -20°C, prior to analyses. The milk energy value was calculated using the equation proposed by Tyrrell and Reid [1965]. Desaturase indices (DI) were estimated according to Garnsworthy *et al*. [2010]. The atherogenicity index was calculated according to Chilliard *et al*. [2003] whereas the thrombogenic index was calculated as described in Ulbricht and Southgate [1991].

The FA proportion in the TMR, powdered root, rumen fluid, and milk samples was analysed based on the method of Szczechowiak *et al*. [2016]. Gas chromatograph (Varian Star CP 3800) fitted with flame ionisation detector and a 100-m fused silica capillary column (i.d. 0.25) coated with 0.2 μm of CP-Sil 88 (CHROMPACK, Varian) was used.

Statistical analysis

The statistical analysis of rumen FA proportion was done based on the mean FA values of the rumen fluid collected from each cow at the 3 sampling time points (before feeding, 3, and 6 h after feeding). The statistical analysis of FA proportion in milk as well as its composition was conducted on mean values of pooled samples from the morning and evening milking. All data were analysed using the general linear model (GLM) for Latin square design according to the following linear model:

$$y_{ijkl} = \mu + c_i + D_j + T_k + e_{ijkl}$$

where:

y_{ijkl} – ijklth observation of fatty acids proportion, μ is the overall mean;
c_i – random effect of ith cow;
D_j – fixed effect of jth diet supplementation (Control, SO1 and SO2);
T_k – fixed effect of kth experimental cycle;
e_{ijkl} – residual connected with ijklth observation.

All pairwise multiple-comparisons were run using the Tukey's test.

Results and discussion

Effect of *Saponaria officinalis* root on milk yield and composition

In the present study, the use of SO increased milk yield (P≤0.05 for SO2; Tab. 2). Furthermore, the SO1 and SO2 diets increased yield of milk composition (protein, lactose, energy) and energy yield (P≤0.05). The effect on milk yield and composition was expected since SO root is rich in polysaccharides (about 31% of DM) that in turn increase the energy supply. A similar result was published by Knowlton *et al*. [1998], who reported an increase in milk yield after providing easily accessible energy (starch). High-calculated energy intake causes a decrease in the number of mobilised adipose tissues during lactation. Other authors observed quadratic effects of dietary sugar on milk yield [Broderick and Radloff 2004]. In order to increase milk yield, the optimal sugar concentration was approximately 5% of the DM basis. The concentration of dietary polysaccharides in our study was low (0.7% and 1.1% in DM basis of the SO1 and SO2 diets, respectively) and it indicates that polysaccharides are not alone in stimulating milk yield and milk composition. Szczechowiak *et al*. [2013] described a positive impact of dietary supplementation with SO on volatile fatty acids (VFA) concentration. VFA elevation improved organic matter digestibility, and finally the utilization of dietary feed ingredients. In the current study we also observed increase of the total VFA concentration (115, 120, 122 mmol/L in Control, SO1 and SO2, respectively) therefore higher milk yield could be expected. It can be suggested that SO addition may improve the energy balance.

Table 2. Effect of two dosages of *Saponaria officinalis* root on the milk yield and composition

Item	Control	SO1	SO2
Milk yield (kg)	25.90^{b}	26.70^{ab}	27.50^{a}
Fat (%)	3.71^{b}	3.87^{ab}	3.94^{a}
Crude protein (%)	2.92^{b}	3.09^{a}	3.11^{a}
Lactose (%)	4.27^{b}	4.52^{a}	4.56^{a}
Total Solids (%)	10.90^{b}	11.48^{a}	11.61^{a}
SNF (%)	7.20^{b}	7.62^{a}	7.67^{a}
Energy (Mcal Kg^{-1})	0.64^{b}	0.68^{a}	0.68^{a}
Energy yield (Mcal)	16.6^{b}	18.0^{a}	18.8^{a}
Fat (g/day)	965^{b}	1032^{a}	1081^{a}
Crude protein (g/day)	758^{b}	826^{a}	854^{a}
Lactose (g/day)	1110^{b}	1209^{a}	1251^{a}

Within a row means of different superscripts differ at P≤0.05.
SO1 – 440 g of *Saponaria officinalis* root supplementation; SO2 – 660 g of *Saponaria officinalis* root supplementation.

Effect of *Saponaria officinalis* root on rumen fatty acid proportion

Biohydrogenation is a defense mechanism against polyunsaturated fatty acids (PUFA) toxicity towards the rumen bacteria. Dietary PUFAs are intensively isomerized by bacterial enzymes and the CLA production is stimulated. The CLA is next reduced to

trans-11 C18:1 acid (VA) and finally to stearic acid C18:0 (SA) [Shingfield *et al*. 2013]. The isomerization process is spontaneous and that of reduction is energy requiring [Harfoot and Hazlewood 1997]. Thus, factors controlling energy availability for the reductases cause accumulation of the BH intermediates [Miri *et al*. 2013]. In the present study we observed significant increase of VA and a tendency to reduce the SA (Tab. 3). It can be suggested that, due to their antimicrobial properties, saponins reduce the available energy by affecting the activity or growth of microbial population and by slowing the final step of BH [Miri *et al*. 2013]. Despite a general acceptation of saponins' action modifying the ruminal ecosystem by inhibiting the population of rumen ciliate protozoa [Brogna *et al.* 2011, Cieslak *et al.* 2014], some publications report negative effects of saponins on cellulolytic bacteria [Wang *el al*. 2000]. Those findings can explain the decrease in proportion of *cis*-9, *trans*-11 C18:2 in the rumen fluid depending on activity and number of cellulolytic bacteria. However, the mechanism of the saponins' action remains unclear and further *in vivo* investigations are required to identify factors limiting the interaction between saponins and the rumen bacteria.

Table 3. Effect of two dosages of *Saponaria officinalis* root on the rumen fatty acid proportions (g/100 g of total fatty acids)

Item	Control	SO1	SO2
Saturated			
C6:0	0.68	0.73	0.78
C8:0	0.16^{a}	0.11^{b}	0.11^{b}
C10:0	0.60	0.57	0.50
C12:0	0.44	0.48	0.43
C14:0	1.24	1.18	1.30
C16:0	21.61^{a}	19.60^{b}	18.87^{c}
C18:0	46.62	44.90	45.33
Monounsaturated			
cis-9 C14:1	0.55^{a}	0.45^{b}	0.42^{b}
cis-9 C16:1	0.22^{a}	0.16^{b}	0.14^{b}
trans-10 C18:1	3.28^{a}	2.74^{b}	2.46^{b}
trans-11 C18:1	2.12^{b}	2.46^{a}	2.37^{a}
cis-9 C18:1	5.60	5.29	5.37
Polyunsaturated			
cis-9, *cis*-12 C18:2	4.76^{b}	5.03^{b}	5.89^{a}
cis-9, *trans*-11 C18:2	0.77^{a}	0.56^{b}	0.57^{b}
trans-10, *cis*-12 C18:2	0.14	0.13	0.14
cis-9, *cis*-12, *cis*-15 C18:3	0.62	0.76	0.66
Long FA	72.91^{c}	75.11^{b}	76.04^{a}
Medium FA	25.71^{a}	23.51^{b}	22.70^{c}
SFA	72.33	73.51	71.23
UFA	27.67	26.49	28.77
MUFA	19.31	18.40	19.67
PUFA	8.36	8.09	9.10
n-6	5.69^{b}	6.08^{b}	7.11^{a}
n-3	2.61	2.35	2.46
n-6/*n*-3	0.68^{c}	0.74^{b}	0.77^{a}

Within a row means of different superscripts differ at P≤0.05;
SO1 – 440 g of *Saponaria officinalis* root supplementation; SO2 – 660 g of *Saponaria officinalis* root supplementation.

Effect of *Saponaria officinalis* root on milk fatty acid proportion

Due to a growing interest in production of milk enriched with UFA, including CLA, the possibility to modulate the BH in the rumen and *de novo* FA synthesis has been a target for research. In the present study the milk FA proportion was favourably changed (Tab. 4). The FAs incorporated into milk come from two sources: the peripheral circulation and FA synthesis in the mammary secretory cells [Niwinska 2010]. The *de novo* FA synthesis accounts for all C4:0 to C12:0, majority of the C14:0 [ca. 95%], and about 50% of C16:0 secreted in milk, whereas all C18:0 carbon and longer chain FAs are thought to be derived from circulating plasma lipids [Shingfield *et al*. 2013]. Either diet [Szczechowiak *et al.* 2016] or endogenous synthesis from VA [Niwinska 2010] are the main factors that influence the FA proportions of *cis*-9, *trans*-11 C18:2. In the present study, supplementation with the SO root resulted in an increase ($P \leq 0.05$) in the total monounsaturated fatty acids (MUFA) and PUFA and consequently, total

Table 4. Effect of two dosages of *Saponaria officinalis* root on the milk fatty acid proportions (g/100 g of total fatty acids)

Item	Control	SO1	SO2
Saturated			
C6:0	0.97^{a}	0.87^{b}	0.76^{c}
C8:0	1.00^{a}	0.96^{a}	0.73^{b}
C10:0	2.67^{a}	2.64^{a}	1.90^{b}
C12:0	3.55^{a}	3.46^{a}	2.61^{b}
C14:0	12.01^{a}	10.32^{b}	10.50^{b}
C16:0	36.80^{a}	32.32^{b}	31.82^{b}
C18:0	12.22^{b}	11.51^{b}	13.31^{a}
Monounsaturated			
cis-9 C14:1	0.47	0.46	0.46
cis-9 C16:1	1.20^{b}	1.17^{b}	1.32^{a}
trans-10 C18:1	0.53	0.69	0.76
trans-11 C18:1	0.32^{b}	0.42^{a}	0.48^{a}
cis-9 C18:1	17.82^{b}	22.45^{a}	22.62^{a}
Polyunsaturated			
cis-9, *cis*-12 C18:2	2.16^{b}	2.94^{a}	2.86^{a}
cis-9, *trans*-11 C18:2	0.53^{c}	0.73^{b}	0.88^{a}
trans-10, *cis*-12 C18:2	0.07^{c}	0.11^{a}	0.09^{b}
cis-9, *cis*-12, *cis*-15 C18:3	0.23^{b}	0.31^{a}	0.32^{a}
Long FA	39.92^{c}	46.23^{b}	48.21^{a}
Medium FA	55.50^{a}	49.41^{b}	48.42^{b}
SFA	71.48^{a}	64.54^{b}	64.00^{b}
UFA	28.52^{b}	35.46^{a}	36.00^{a}
MUFA	25.00^{b}	30.87^{a}	31.32^{a}
PUFA	3.52^{b}	4.59^{a}	4.68^{a}
n-6	2.55^{b}	3.43^{a}	3.34^{a}
n-3	0.71^{b}	0.82^{a}	0.85^{a}
n-6/*n*-3	0.72^{a}	0.74^{a}	0.70^{b}

Within a row means of different superscripts differ at $P \leq 0.05$.
SO1 – 440 g of *Saponaria officinalis* root supplementation; SO2 – 660 g of *Saponaria officinalis* root supplementation.

UFA, including majority of C18:1 isomers, *cis*-9, *cis*-12 C18:2 and *cis*-9, *cis*-12, *cis*-15 C18:3 FA, as well as CLA; *cis*-9, *trans*-11 C18:2 and *trans*-10, *cis*-12 C18:2. The observed increase in CLA and UFA in the milk fat may be associated with the protozoa population. The protozoa contain greater than bacteria proportion of UFA including CLA [Mao *et al*. 2012]. The presence of protozoa in the rumen may improve the contribution of UFA and CLA for small intestine absorption by ruminants. On the other hand proportion of *cis*-9, *trans*-11 C18:2 and *cis*-9 C18:1 in the milk fat is highly dependent on endogenous FA synthesis in the mammary gland by stearoyl-CoA desaturase (SCD; also referred to as Δ^9-desaturase) [Garnsworthy *et al*. 2010]. The SCD expression in sheep muscles was reduced in response to addition of tea saponins [Mao *et al*. 2012]. In our study we defined the SCD activity indirectly by calculating the DI [Garnsworthy *et al*. 2010]. Since the SO supplementation increased the DI_{C16} and DI_{C18} it can be suggested that SO saponins positively influenced SCD activity and led to increase in the *cis*-9 C16:1 and *cis*-9 C18:1 FA in the milk fat (Tab. 5). Moreover, the DI_{C16} and DI_{C18} were the only DIs affected by saponins thus we cannot exclude a higher affinity of the SCD enzyme for FAs with a longer carbon chain (e.g. 16 and 18 carbon atoms) than those with a shorter carbon chain [Brogna *et al*. 2011]. In our study, lack of changes in DI_{RA} was accompanied by an increase in *cis*-9, *trans*-11 C18:2 and *trans*-11 C18:1. Such phenomenon may result from elevated proportion of the two FAs in milk which in turn may mask the variation in SCD activity.

Table 5. Effect of two dosages of *Saponaria officinalis* root on desaturation (DI), thrombogenic and atherogenicity indexes of the produced milk

Item	Control	SO1	SO2
DI_{C14}	0.038	0.044	0.042
DI_{C16}	0.031^{c}	0.035^{b}	0.040^{a}
DI_{C18}	0.592^{c}	0.661^{a}	0.633^{b}
DI_{RA}	0.538	0.519	0.565
Thrombogenic index	3.87^{a}	2.80^{b}	2.80^{b}
Atherogenicity index	3.17^{a}	2.22^{b}	2.14^{b}

Within a row means of different superscripts differ at $P \leq 0.05$.
SO1 – 440 g of *Saponaria officinalis* root supplementation; SO2 – 660 g of *Saponaria officinalis* root supplementation.

Although we noticed an impact of SO saponins on FA proportion in milk, some earlier studies did not report such effects [Brogna *et al*. 2011, Mao *et al*. 2012]. These discrepancies, however, may suggest that ability of plant secondary metabolites to modify the lipid metabolism and enzyme activity most probably results from multiple interactions of the diet and bioactive compound (type and dose rate). As far as the rumen FAs are concerned, more precise investigation of mechanisms regulating interaction of saponin and the proportion of milk FA are required.

In the present study the total SFA proportion was reduced whereas that of UFA, MUFA and PUFA increased (Tab. 4). Such situation may be related to the reduction of

the thrombogenic and atherogenic indexes in response to SO treatment (Tab. 5). Milk and dairy products are main sources of two SFAs – C12:0 and C14:0 in the human diet [Givens *et al*. 2006]. These FAs are associated with increased risk of cardiovascular disease. Reduction of thrombogenic and atherogenic indexes means that the nutritional and health quality of the produced milk increases. Unlike in the present study, only minor changes in SFA were observed after diet supplementation with saponin extract from *Yucca schidigera* [Benchaar and Chouinard 2009]. Thus, the source of saponins (steroidal *vs* triterpenoid) may be also important to their activity in a host animal. The health-promoting properties of milk produced by cows fed powdered SO root result from the increased proportion of *cis*-9, *cis*-12, *cis*-15 C18:3. This FA shows ability to desaturate and elongate to *n*-3 PUFA (e.g. C22:6 *n-3*; C22:5 *n-3*) in human tissues [Molendi-Coste *et al*. 2011]. Despite a low potential of *cis*-9, *cis*-12, *cis*-15 C18:3 for metabolic conversion to *n*-3 PUFA [Givens 2010], it exerts several, health promoting activities in humans and is considered dietary essential [Molendi-Coste *et al*. 2011].

Supplementation of dairy cow diets with two dosages of SO powdered root (440 g/day and 660 g/day) influenced rumen FA proportion and improved the milk FA proportion without negative effect on milk yield and composition. Both SO dosages seem to effectively modulate milk FA proportion in dairy cows by altering the SCD activity in the mammary gland. Changes in the milk fat FAs, particularly in case of the higher SO dosage (660 g/day) were not proportional to the dosage applied due to a lack of unfavorable changes in other parameters, including milk basic composition. Therefore, we recommend the SO dosage of 440 g/day as dietary supplement for dairy cows.

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REFERENCES

1. AOAC 2007 – *Official Methods of Analysis*, 18th edn. Association of Official Analytical Chemists, Gaithersburg, MD.
2. BENCHAAR C., CHOUINARD P.Y., 2009 – Short communication - Assesment of the potential of cinnamaldehyde, condensed tannins, and saponins to modify milk fatty acid composition of dairy cows. ***Journal of Dairy Science*** 92, 3392-2296.
3. BRODERICK G. A., RADLOFF W. J., 2004 – Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. ***Journal of Dairy Science*** 87, 2997-3009.
4. BROGNA D. M. R., NASRI S., BEN - SALEM H., MELE M., SERRA A., BELLA M., PRIOLO A., MAKKAR H. P. S., VASTA V., 2011 – Effect of dietary saponins from *Quillaja saponaria* L. on fatty acid composition and cholesterol content in muscle *Longissimus dorsi* of lambs. ***Animal*** 5, 1124-1130.

5. CIESLAK A., SZUMACHER-STRABEL M., STOCHMAL A., OLESZEK W., 2013 – Plant components with specific activities against rumen methanogens. ***Animal*** 7[2], 253-265.
6. CIESLAK A., ZMORA P., STOCHMAL A., PECIO L., OLESZEK W., PERS-KAMCZYC E., SZCZECHOWIAK J., NOWAK A., SZUMACHER - STRABEL M., 2014 – Rumen antimethanogenic effect of *Saponaria officinalis* phytochemicals *in vitro*. ***Journal of Agricultural Science*** 152, 981-993.
7. CHILLIARD Y., FERLAY A., ROUEL J., LAMBERETT G., 2003 – A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. ***Journal of Dairy Science*** 86, 1751-1770.
8. GARNSWORTHY P.C., FENG S., LOCK A.L., ROYAL M.D., 2010 – *Short communication:* Heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. ***Journal of Dairy Science*** 93, 1743-1748.
9. GIVENS D.I., SHINGFIELD K.J., 2006 – Optimising dairy milk fatty acid composition. In - Williams C.M., Buttriss J. [ed.], Improving the fat content of foods. Wood head Publishing Ltd, Cambridge, 252-280.
10. GIVENS D.I., 2010 – Milk and meat in our diet: good or bad for health? ***Animal*** 4,1941-1952.
11. HARFOOT C. G., HAZLEWOOD G. P., 1997 – Lipid metabolism in the rumen. Pages 382-426 in The Rumen Microbial Ecosystem. 2nd ed. P. N. Hobson, ed. Elsevier, London, UK.
12. INRA IZ, Instytut Zootechniki – 1993, Normy żywienia bydła, owiec i kóz [Feed requirements for cattle, sheep and goats]. Omnitech Press, Warsaw, Poland.
13. JÓŹWIK A., STRZAŁKOWSKA N., BAGNICKA E., ŁAGODZIŃSKI Z., PYZEL B., CHYLIŃSKI W., CZAJKOWSKA A., GRZYBEK W., SŁONIEWSKA D., KRZYŻEWSKI J., HORBAŃCZUK J.O., 2010 – The effect of feeding linseed cake on milk yield and milk fatty acid profile in goats. ***Animal Science Papers and Reports*** 28 (3), 245-251.
14. JÓŹWIK A., STRZAŁKOWSKA N., MARKIEWICZ-KĘSZYCKA M., KRZYŻEWSKI J., LIPIŃSKA P., RUTKOWSKA J., WRÓBLEWSKA B., KLUSEK J., COOPER R.G., 2016 – Effects of replacing rapeseed cake with linseed cake in a corn-grass silage-based diet for milking cows. ***Animal Science Papers and Reports*** 34 (2), 129-142.
15. KNOWLTON K.F., DAWSON T.E., GLENN B.P., HUNTINGTON G.B., ERDMAN R.A., 1998 – Glucose metabolism and milk yield of cows infused abomasally or ruminally with starch. ***Journal of Dairy Science*** 81, 3248-3258.
16. KOBA K., YANAGITA T., 2013 – Health benefits of conjugated linoleic acid [CLA]. ***Obesity Research and Clinical Practice*** 8 (6), 525-532.
17. MAO H.L., WANG J.K., LIN J., LIU J.X., 2012 – Fatty acid profiles and stearoyl-CoA desaturase gene expression in *Longissimus dorsi* muscle of growing lambs influenced by addition of tea saponins and soybean oil. ***Asian-Australasian Journal of Animal Sciences*** 2012, 25(5), 648-652.
18. MIRI V.H., TYAGI A.K., EBRAHIMI S.H., MOHINI M., 2013 – Plant extract enhanced ruminal CLA concentration, *in vitro*. ***Journal of Animal and Feed Sciences*** 22, 219-228.
19. MOLENDI-COSTE O., LEGRY V., LECLERCQ I.A., 2011 – Review article: Why and how meet n-3 PUFA dietary recommendations? ***Gastroenterology Research and Practice*** doi:10.1155/2011/364040.
20. NIWINSKA B., 2010 – Endogenous synthesis of rumenic acid in humans and cattle. ***Journal of Animal and Feed Sciences*** 19, 171-182.
21. SHINGFIELD K. J., BONNET M., SCOLLAN N.D., 2013 – Recent developments in altering the fatty acid composition of ruminant-derived foods. ***Animal*** 7, 132-162

22. SZCZECHOWIAK J., SZUMACHER-STRABEL M., STOCHMAL A., NADOLNA M., PERS-KAMCZYC E., NOWAK A., KOWALCZYK M., CIESLAK A., 2013 – Effect of *Saponaria officinalis* L. or *Panax ginseng* C.A Meyer triterpenoid saponins on ruminal fermentation *in vitro*. ***Annals of Animal Science*** 13, 815-827.
23. SZCZECHOWIAK J., SZUMACHER-STRABEL M., EL-SHERBINY M., PERS-KAMCZYC E. , PAWLAK P., CIESLAK A., 2016 – Rumen fermentation, methane concentration and fatty acid proportion in the rumen and milk of dairy cows fed condensed tannin and/or fish-soybean oils blend. ***Animal Feed Science and Technology*** 216, 93-107.
24. SZKUDELSKA K., SZUMACHER-STRABEL M., SZCZECHOWIAK J., BRYSZAK M., PERS-KAMCZYC E., STOCHMAL A., CIESLAK A., 2016 – The effect of triterpenoid saponins from *Saponaria officinalis* on some blood hormones, metabolic parameters and fatty acid composition in dairy cows. ***Journal of Agricultural Science*** 154, 532-541.
25. STRZAŁKOWSKA N., JÓŹWIK A., BAGNICKA W., KRZYŻEWSKI J., HORBAŃCZUK K., PYZEL B., HORBAŃCZUK J.O., 2009 – Chemical composition, physical traits and fatty acid profile of goat milk as related to the stage of lactation. ***Animal Science Papers and Reports*** 27 (4), 311.320.
26. TYRRELL H.F., REID J.T., 1965 – Prediction of the energy value of cow's milk. ***Journal of Dairy Science*** 48, 1215-1223.
27. ULBRICHT T.L.V., SOUTHGATE D.A.T., 1991 – Coronary heart disease - seven dietary factors. ***Lancet*** 338, 985-992.
28. VAN SOEST P.J., ROBERTSON J.B., LEIS B.A., 1991 – Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. ***Journal of Dairy Science*** 74, 3583-3597.
29. WANG Y., MCALLISTER T.A., YANKE L.J., CHEEKE P.R., 2000 – Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. ***Journal of Applied Microbiology*** 88, 887-896.
30. ZYMON M., STRZETELSKI J., SKRZYNSKI G., 2014 -–Aspects of appropriate feeding of cows for production of milk enriched in the fatty acids, EPA and DHA. ***Journal of Animal and Feed Sciences*** 23, 109-116.