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GOAT YOGHURT DRINKS WITH ELEVATED α-LINOLENIC ACID CONTENT AND ENRICHED WITH YACON FIBER

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ABSTRACT

Goat milk and goat milk products are very valuable in human nutrition because of their favorable nutrient composition which can be further boosted by the addition of prebiotic fiber and probiotic bacteria. It has also been possible to change the fatty acid profile of goat milk through feed composition. The aim of this study was to increase the nutritional value of goat milk by producing a probiotic yoghurt drink made from milk with elevated omega-3 fatty acids and enriched with natural yacon prebiotics. Goat nutrition is one of the key factors how we can naturally increase omega-3 fatty acid content in goat milk. In our study, twenty four White Shorthair goats were divided into the control and experimental group which was supplemented with 55 mL of linseed oil per day for eight weeks to increase the monounsaturated and polyunsaturated fatty acid content in the milk. The yoghurt milk drinks were formulated from individual goat milk samples with added bifidobacteria and yacon prebiotics. Our results showed that goat feed supplementation with linseed oil indeed positively changed fatty acid profile of goat milk in which α-linolenic acid content increased while, at the same time, lauric, myristic and palmitic acid contents decreased. Also, yoghurt drinks enriched with yacon prebiotics have shown higher bifidobacteria counts compared to the control.

Keywords: fatty acid; goat milk; α-linolenic acid; linseed oil; yacon

INTRODUCTION

In the last few years, the number of goat farms (in particular operating under organic farming practices) has been increasing not only in the Czech Republic but also worldwide leading to greater production of goat milk **(Josrová, 2018)**. Therefore, farmers and dairy producers are actively seeking to develop new products and formulas which would attract consumers' attention. Goat milk represents a welcome alternative to products made from cow's milk. For instance, products made from goat milk are considered to be healthier because goat milk shows better nutritional characteristics like digestibility due to smaller diameter of fat globules (3.5 μm in goat milk compared to 4.5 μm in cow's milk) which causes the fat to be better dispersed in the milk **(Park et al., 2007)**. Goat milk contains also a higher amounts of caproic, caprylic and capric acids (in total 15 – 18% compared to 5 – 9% in cow's milk) that were shown to be beneficial to humans **(Sanz Sampelayo et al., 2007)**. These short-chain fatty acids have been proven to help patients with malabsorption syndrome, intestinal disorders, coronary heart diseases, cystic fibrosis and other conditions **(Haenlein, 2004; Ribeiro and Ribeiro, 2010)**. Furthermore, it is possible to increase the content of omega-3 fatty acids in goat milk by supplementing regular goat feed with moderate amounts of linseed oil which is rich in α-linolenic acid as described in **Borková et al. (2018)**. Differences in milk casein content were also found to impact human health. Goat α_{S1}-casein content is lower (typically 10 – 13% of total casein content) compared to cow's milk and depends on the particular genetic variant for α_{S1}-casein. The presence of null alleles for α_{S1}-casein such as 0_1, 0_2, 0_4 and N cause the absence of α_{S1}-casein in goat milk **(Caboni et al., 2016)** which is an important factor in decreasing the allergic sensitation of α_{S1}-casein **(Ballabio et al., 2011; Hochwallner et al., 2014)**.

Many more possibilities are available to boost nutritional impact of goat milk products as using milk high in omega-3 essential fatty acids, probiotic bacteria and prebiotics. Probiotic bacteria are well known for their antimicrobial, antioxidant and immunomodulatory effects **(Lee et al., 2011; Tomáška et al., 2015)**. Prebiotics are more or less indigestible components of foods comprising fructooligosaccharides (FOS) which positively influence the growth and activity of probiotic bacteria in the gastrointestinal system **(Valcheva and Dieleman, 2016)**. FOS are oligosaccharides consisting of D-fructose chains connected by β-(1→2) glycosidic bonds and capped by glucose. The yacon (*Smallanthus sonchifolius*) plant belongs to the *Asteraceae* family and grows in eastern Andes from Venezuela to northern Argentina **(Caetano et al., 2016)**. Yacon tuber is a great source of FOS **(Topolska**

et al., 2015) which do not undergo hydrolysis by human intestinal enzymes and are instead selectively fermented by bacteria in the colon leading to a more balanced composition of gut microbiota. Dry yacon tubers contain up to 50% (very rarely up to 70%) of FOS **(Velez et al., 2013)**.

The aim of this study was to increase the nutritional value of goat milk by producing a probiotic yoghurt drink made from goat milk with elevated omega-3 fatty acids and enriched with natural yacon prebiotics.

Scientific hypothesis

This research aimed to confirm the fact that milk from goats supplemented with linseed oil can be successfully used to produce dairy products with elevated polyunsaturated fatty acids. A special interest was paid to the use of natural yacon prebiotics to elevate the numbers of bifidobacteria in such products to increase their health benefits.

MATERIAL AND METHODOLOGY

Animals, animal diet, feed analysis

The experiment was carried out on a private organic farm near Liberec (Czech Republic) using the following set-up: Twenty four White Shorthaired dairy goats (on their third lactation) were selected based on age (3 years), date of kidding (between March and April 2017) and litter size (2 kids) and divided into experimental (LO) and control (C) groups, each comprising twelve animals. The goats were housed indoors and their feed consisted of: hay (*ad libitum*), haylage (3 kg per animal per day) and grain mix of 50% corn, 25% barley and 25% oat (1 kg per animal per day). Goats in the LO group were fed basic ration supplemented with 55 mL of linseed oil (LO) per animal per day and the C group was fed the same diet without LO supplement. The LO supplementation begun on May 17 and lasted for eight weeks till July 11, 2017. LO was supplied by 1. zemědělská a.s. (Chorušice, Czech Republic).

The animal feed was sampled on July 11, 2017 and analyzed according to methods published in **Regulation (EC) No 152/2009** for dry matter, ash, crude protein, ether extract and crude fiber. The fatty acid analysis in feed including lipid extraction was carried out as described by **Kubelková et al. (2013)**. Results are given in Table 1.

Milk sampling and analysis

Individual milk yield measurements and milk analyses were carried out at three time points – at the beginning (week 0), in the middle (week 4) and at the end (week 8) of the experiment. Results are shown in Table 2. Milk samples were analyzed for fat, protein and total solids by IR milk analyzer DairySpec FT (Bentley Instruments). The instrument has been calibrated using a set of goat milk samples which were simultaneously analyzed by reference methods in accredited laboratory (MILCOM a.s., Prague).

Two pooled milk samples were created to mimic standard practices on the farm. Pooled samples were created from individual milk samples (taken on July 11, 2017) obtained from the C and LO goat groups. One pooled sample was created from milk in the control group (to produce one PCY drink) and another one from milk in the experimental group fed linseed oil (to produce one PLY drink).

The fat in yoghurt drinks was extracted according to **ČSN EN ISO 1211 (2011)**. Fatty acids (FA) were then re-esterified into the corresponding methyl esters (FAME) and were analyzed by gas chromatography according to method described by **Borková et al. 2018**. FAME were identified using external analytical standard (Supelco, USA). The content of a particular fatty acid was calculated as a ratio of its peak area.sum^{-1} of peak areas of all fatty acids and given in g.100 g^{-1} total fatty acids.

Yoghurt drink formulation

Milk samples for yoghurt drinks were taken the last day of the experiment (July 11, 2017) during morning milking. Twelve yoghurt drinks (denoted as ILY) from the group fed linseed oil and twelve yoghurt drinks (denoted as ICY) originating from the control group were individually produced. All these yoghurt drinks in both groups contained 5 g of yacon tuber powder (see below). Also, twelve individual yoghurt drinks were prepared from the group fed linseed oil but without the addition of yacon tuber powder (denoted as IL) to enable the investigation of yacon powder addition on bacterial growth. Both yoghurt drinks made from pooled samples (PLY or PCY) contained each 5 g of yacon tuber powder.

Yoghurt drinks were prepared as follows: 5 g of lyophilized Peruvian yacon tuber powder (Gloobe corp., Czech Republic) were added into 95 g of goat milk, pasteurized at 84 °C for 10 min and fermented using 0.1% CCDM 528 (*Streptococcus thermophilus* and *Lactobacillus delbruckii* ssp. *Bulgaricus*, Laktoflora®) and 1% *Bifidobacterium animalis* ssp. *lactis* (Bb12, Chr. Hansen) at 30 °C for 16 – 18 h.

Yoghurt bacteria counts (in colony forming units, CFU) were estimated according to **ČSN ISO 7889 (2004)**, bifidobacteria counts (in CFU) according to **ČSN ISO 29981 (2010)** and FOS analysis as described in **Bohačenko and Pinkrová (2014)**.

Statisic analysis

The data were analyzed in Statistica (ver. 12, StatSoft). Milk yield and milk composition were tested by repeated measures ANOVA. The FA content, the numbers of CFU of yogurt bacteria and bifidobacteria were tested by one-way ANOVA. Tukey's *post-hoc* HSD test (p <0.05) was used to evaluate differences between groups. Results are expressed as mean value with the standard error of mean (*SEM*).

RESULTS AND DISCUSSION

Our previous work **(Borková et al., 2018)** revealed that feed supplemented either with linseed oil or linseed extrudate increased the amounts of omega-3 fatty acids in goat milk which can be successfully used to produce yoghurt drinks with added value. We found out that feed supplemented with linseed oil yields better results than linseed extrudate. Therefore, we decided to modify the previous experiment using feed supplementation with linseed oil to get enriched omega-3 goat milk which was used to produce yoghurt drinks with added yacon prebiotics to boost the drink's health benefits. We also

Table 1 Composition of basic diet and linseed oil.

	Hay	Haylage	Grain mix	Linseed oil
DM (g.100 g^{-1} FW)	78.8	51.3	87.5	–
Crude protein (g.100 g^{-1} DM)	6.26	12.9	9.88	–
Ether extract (g.100 g^{-1} DM)	1.40	2.49	2.48	–
Crude fibre (g.100 g^{-1} DM)	42.7	23.2	7.75	–
Ash (g.100 g^{-1} DM)	6.42	9.11	13.7	–
FA (g.100 g^{-1} FA):				
SFA	34.9	20.3	23.0	9.58
MUFA[6]	34.4	14.8	19.5	16.0
PUFA[7]	30.7	64.9	57.5	74.4
n-6 FA	16.9	34.2	38.6	15.5
n-3 FA	13.8	30.0	18.9	58.9
ALA[8]	12.4	28.6	17.5	58.9

Note: DM = dry matter; FW = fresh weight; Crude protein = nitrogen content × 6.25; Ether extract = crude fat; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid, ALA = α-linolenic acid.

Table 2 Milk yield and milk composition.

	C	LO	*SEM*	*p* value
Milk yield, 0 week (kg.day^{-1})	1.25	1.38	0.11	NS
Milk yield, 4 week (kg.day^{-1})	1.03^{b}	1.62^{a}	0.12	$p < 0.05$
Milk yield, 8 week (kg.day^{-1})	1.13^{b}	1.56^{a}	0.10	$p < 0.05$
Milk fat, 0 week (g.100 g^{-1})	3.59	3.23	0.16	NS
Milk fat, 4 week (g.100 g^{-1})	3.07	3.08	0.09	NS
Milk fat, 8 week (g.100 g^{-1})	2.81	2.84	0.11	NS
Milk protein, 0 week (g.100 g^{-1}	3.18	3.05	0.06	NS
Milk protein, 4 week (g.100 g^{-1})	2.98	3.01	0.05	NS
Milk protein, 8 week (g.100 g^{-1})	2.93	2.92	0.04	NS
Total solids, 0 week (g.100 g^{-1})	12.0	11.7	0.21	NS
Total solids, 4 week (g.100 g^{-1})	11.1	11.2	0.13	NS
Total solids, 8 week (g.100 g^{-1})	10.7	10.9	0.16	NS

Note: NS = not found to be significantly different ($p > 0.05$); C = goats in the control group (N = 12); LO = goats fed linseed oil (N = 12); *SEM* = standard error of mean.

increased the length of linseed oil supplementation to goats to eight weeks to prove that it has a long-lasting effects on omega-3 fatty acid elevation (and other quality indicators) in goat milk. Our last experiment published in 2018 found a minor (but statistically insignificant) increase in milk yield, milk fat and total milk solids in milk samples from goats fed linseed oil. Results from the current study revealed that after four weeks of linseed oil supplementation the milk yield has increased ($p < 0.05$) and this effect lasted until the end of experiment. The linseed oil supplementation did not affect other quality parameters like fat and protein content or total solids (Table 2). Thus, the increased milk yield and quality (see below) after linseed oil supplementation may positively impact farm's economy offsetting the costs for feed supplementation.

From Table 3 (listing some major and nutritionally important minor fatty acids) it is evident that linseed oil supplementation (ILY) changes fatty acid profile in the final product. To sum up the results, saturated fatty acid (SFA) levels were significantly decreased ($p < 0.001$) while the levels of monosaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) increased ($p < 0.01$, $p < 0.001$ resp.). Contrary to our 2018 study, linseed oil supplementation was able to reduce short-chain SFAs and C12:0 in yoghurt drinks (in particular C6:0, $p < 0.05$; C8:0, $p < 0.05$; C10:0, $p < 0.01$; C12:0, $p < 0.05$). These are interesting results because the data in scientific papers so far either found an increase of short-chain SFA levels as in **Bernard et al. (2009)** or no change at all **(Martínez Marín et al., 2011)** after linseed oil supplementation. In the case of medium-chain SFAs (C14:0 and C16:0), we saw their levels to decrease ($p < 0.01$) after linseed oil supplementation which is in accordance with the data published in scientific literature **(Bernard et al., 2009; Martínez Marín et al., 2011)** and our last paper **(Borková et al., 2018)**. According to **Martínez Marín et al. (2011)** the observed reduction of short and medium-chain SFAs was likely caused by the increased intake of PUFAs from linseed oil added to the feed which might have affected some crucial enzymes in the *de novo* fatty acid synthesis pathway such as acetyl-CoA carboxylase and fatty acid synthase. The *de novo* synthesis in the mammary gland is the major source of fatty acids with less than sixteen carbon atoms. Unlike our previous short-term supplementation experiment, the long-term supplementation with linseed oil decreased the levels of C14:0, C16:0 and also the levels of C6:0 to C12:0.

The increased amounts of *trans*-C18:1 and *trans*-C18:2 isomers, ALA and conjugated linoleic acid contributed to the elevated levels of MUFAs and PUFAs. These results are in accordance with findings of **Martínez Marín et al. (2011) and Bernard et al. (2009)** who also observed such increases after linseed oil supplementation. The increase of the unsaturated fatty acids mentioned above in yoghurt drinks was caused by the ALA present in linseed oil. Fatty acids longer than eighteen carbons get into milk from lipoproteins present in blood plasma (which themselves originate from food or animal's fat depots). The PUFAs present in feed undergo biohydrogenation in rumen during digestion which leads to the formation of C18:0 and other isomers like *t*11-C18:1 (vaccenic acid) and *t*11,*c*15-C18:2 **(Chilliard et al., 2007)**. These fatty acids are subsequently absorbed in the colon and transported through blood into the mammary gland where they might be desaturated by stearoyl-CoA desaturase activity. Contrary to our previous report in **Borková et al. (2018)**, the linseed supplementation did not decreased *c*9-C18:1 in yoghurt drinks. The PUFAs biohydrogenation in goat rumen is a very complex process influenced by many external and internal factors. The composition of metabolites resulting from biohydrogenation process is dependent on the supplement type and feed itself which both impact the lipogenesis in the mammary gland and the activity of key enzymes. Unlike our previous experiment, we did not see the desaturase activity to drop significantly in the yoghurt drinks (see the desaturase index in Table 3) made from the experimental group (ILY). This fact is the likely cause why we did not observe the *c*9-C18:1 fatty acid to decrease. It is worth to mention that the present study confirmed again that ALA levels in yoghurt drinks remained increased.

From the nutritional perspective it is important to underscore the increased content of *trans*-fatty acids in the ILY drinks. As a matter of fact, *trans*-fatty acids in food can negatively influence consumers' health. However, this notion is not true for all *trans*-fatty acids **(Anadón et al., 2010; Jacome-Sosa et al., 2014; Ganguly and Pierce, 2015)** like vaccenic acid (*t*11-C18:1) which is the main biohydrogenation intermediate of ALA.

All our yoghurt drinks (with or without the addition of yacon tuber powder) made from enriched omega-3 milk (LO goat group) have met the requirements of the **Regulation no. 397/2016 Col. of Czech Republic** specifying that all yoghurt products must contain at least 7 log CFU.g^{-1} (which equals to 10^7 CFU.g^{-1}). Yoghurt drinks containing yacon tuber powder (ILY) showed significant increase in the number of bifidobacteria than yoghurt drinks without the yacon addition (IL) (Table 4). This result is likely due to the presence of fructooligosaccharides in the yoghurt drinks which are a welcomed substrate for the probiotic bacteria leading to their increased counts.

Two pooled milk samples (one from the experimental, the other from control group) were created to mimic standard practices on the farm because farmers usually do not have access to individual milk samples but only to the pooled milk from the herd. Both pooled samples were used to manufacture yoghurt drinks with yacon tuber powder (one drink per each pooled sample denoted as PLY and

Table 3 Fatty acid composition of goat yoghurt drinks.

FA (g.100 g^{-1} total FA).	ICY	ILY	*SEM*	*p* value
C4:0	2.43	2.60	0.051	NS
C6:0	2.52^a	2.30^b	0.055	p <0.05
C8:0	2.62^a	2.25^b	0.078	p <0.05
C10:0	8.92^a	7.33^b	0.303	p <0.01
C12:0	3.84^a	3.26^b	0.121	p <0.05
C14:0	10.4^a	9.20^b	0.224	p <0.01
C16:0	27.0^a	23.8^b	0.573	p <0.01
C18:0	10.6	11.2	0.389	NS
t-C18:1	2.10^b	4.91^a	0.386	p <0.001
*c*9-C18:1	19.1	18.9	0.408	NS
t-C18:2	0.89^b	2.73^a	0.231	p <0.001
*c*9,*c*12-C18:2	2.13	2.32	0.070	NS
*c*9,*c*12,*c*15-C18:3 (ALA)	1.00^b	1.53^a	0.078	p <0.001
CLA	0.56^b	1.17^a	0.082	p <0.001
*c*9-C18:1/C18:0	1.82	1.77	0.084	NS
SFA	71.5^a	64.8^b	0.997	p <0.001
MUFA	23.6^b	27.1^a	0.620	p <0.01
PUFA	4.91^b	8.10^a	0.425	p <0.001
n-6 FA	2.32	2.55	0.072	NS
n-3 FA	1.13^b	1.66^a	0.077	p <0.001

Note: Different small caps in the superscript indicate differences between groups (at p <0.05), NS = not significantly different (at p >0.05); ICY = control group (N = 12); ILY = goats fed linseed oil (N = 12); *SEM* = standard error of mean; *t*-C18:1 = *trans* isomers C18:1 including e.g. vaccenic acid (*t*11-C18:1); *t*-C18:2 = *trans* isomers C18:2 including e.g. *t*11,*c*15-C18:2; ALA = α-linolenic acid; CLA = conjugated linoleic acid (mixture of isomers *c*9,*t*11-C18:2 and *t*9,*c*11-C18:2); *c*9-C18:1/C18:0 = desaturase index; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

Table 4 Comparison of the number of colony forming units in yoghurt drinks.

	ILY	IL	*SEM*	*p* value
∑ yoghurt bacteria (log CFU.g^{-1})	8.35	8.50	0.056	NS
bifidobacteria (log CFU.g^{-1})	6.96^{a}	6.80^{b}	0.033	p <0.05

Note: Different small caps in the superscript indicate differences between groups (at p <0.05); NS = not significantly different (at p >0.05); ILY = yoghurt drinks with yacon tuber powder from goats fed linseed oil (n = 12); IL = yoghurt drinks without yacon tuber powder from goats fed linseed oil (n = 12); *SEM* = standard error of mean.

Table 5 Composition of yoghurt drinks made from pooled samples.

	PCY	PLY
Fructooligosaccharides (g.100 g^{-1})	0.73	0.81
∑ yoghurt bacteria (log CFU.g^{-1})	8.56	8.51
bifidobakteria (log CFU.g^{-1})	7.04	6.88
Fatty acids (g.100 g^{-1} total FA)		
C4:0	2.59	2.41
C6:0	2.56	2.30
C8:0	2.61	2.28
C10:0	9.03	7.42
C12:0	3.90	3.24
C14:0	10.6	9.09
C16:0	27.3	23.1
C18:0	10.2	11.7
t-C18:1	2.03	5.20
*c*9-C18:1	18.9	18.7
t-C18:2	0.87	2.85
*c*9,*c*12-C18:2	2.08	2.35
*c*9,*c*12,*c*15-C18:3 (ALA)	0.96	1.59
CLA	0.55	1.17
SFA	71.9	64.4
MUFA	23.3	27.3
PUFA	4.78	8.32
n-6 FA	2.26	2.59
n-3 FA	1.10	1.71

Note: PCY = yoghurt drinks with yacon tuber powder and bifidobacteria from control group (n = 1); PLY = yoghurt drinks with yacon tuber powder and bifidobacteria from experimental group (goats fed linseed oil) (n = 1); *t*-C18:2 = *trans* isomers C18:2 including e.g. *t*11, *c*15-C18:2; ALA = α-linolenic acid; CLA = conjugated linoleic acid (mixture of isomers *c*9, *t*11-C18:2 and *t*9, *c*11-C18:2); SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

PCY) because we were interested if there is any difference in fatty acid profile in yoghurt drinks made from the pooled sample versus yoghurt drinks made from individual milk samples. The results for both groups are shown in Table 5. The yoghurt drinks made from pooled milk samples from goats supplemented with linseed oil (PLY) had increased contents of MUFAs, PUFAs and a lower content of SFAs similar to findings for ILY yoghurt drinks. PLY yoghurt drinks had 66% higher ALA content (compared to the PCY group used as a control). The same comparison for yoghurt drinks from individual milk samples (ILY and ICY) has shown only 53% increase in ALA content. The higher amount of ALA in the pooled samples (66%) was caused by some goats having higher milk yield with the highest ALA content (unpublished results). Fructooligosaccharide content in yoghurt drinks from the pooled samples was 0.73 – 0.81 g.100 g^{-1} of yoghurt drink thus these yoghurt drinks can be a valuable source of prebiotics, probiotics and α-linolenic acid.

CONCLUSION

Goat yoghurt drinks with bifidobacteria and yacon tuber powder are novel products that might be interesting for health conscious consumers providing them with added value. These drinks are a good source of fructooligosaccharides and bifidobacteria compared to regular products without any added prebiotics. In case of the milk produced by goats fed linseed oil it is possible to manufacture a product which has an increased omega-3 fatty acid content and decreased content of saturated fatty acids. These yoghurt drinks eventually contained significantly higher amounts of α-linolenic acid and lower levels of lauric, myristic and palmitic acid.

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