

Effect of inclusion of bakery by-products in the dairy cow's diet on milk fatty acid composition

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Research Article

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Author for correspondence:Ratchaneewan Khiaosa-ard, Email: ratchaneewan.khiaosa-ard@vetmeduni.ac.at**Abstract**

Bakery by-products (BP), rich in fats and sugars, are unconventional feed sources for cows whose effects on milk fat composition have not yet been evaluated. This research paper aimed to assess the effects of dietary BP inclusion rate and feeding period on the milk fatty acid composition. Twenty-four Simmental cows were fed a diet without BP (CON) for 1 week. Then they either continued with the CON diet or switched to one of the BP diets (with 15% or 30% BP in diet dry matter) for 3 weeks. Milk samples were taken before diet change and three times during BP feeding and analysed for fatty acid composition. Data showed that increasing BP content in the diet increased total fatty acid intake, especially of 18:1 n9. In the milk fat, the percentages of total monounsaturated fatty acids especially of the 18:1 origin linearly increased with increasing dietary BP level. The percentage of fatty acids de novo synthesized in the mammary gland (the sum of 4:0–14:0) remained similar among diets (32–34% of total fatty acids). The 16:0 percentage dropped from 32.5 to 29.6% and from 33.6 to 28.3% for 15% and 30% BP, respectively. Only 30% BP elevated the percentage of conjugated linoleic acids (CLA: by 59%) compared with CON throughout the 3 weeks. Proportions of 18:2 n6 and 18:3 n3 and the n6:n3 ratio were unaffected by BP and feeding time. BP feeding improved all those estimated health indices of the milk fat that are suggested to be related to coronary health. In summary, the inclusion of BP in dairy rations beneficially shifted the milk fatty acid profile to more 18:1 fatty acids at the expense of 16:0. At a 30% inclusion rate, BP feeding showed an additional benefit of increased CLA content in milk fat.

Bakery by-products (BP) are recycled as animal feed. BP include various bakery leftovers like bread, biscuit, cookies and dough that are unsold and deemed to have expired. BP holds great value as energy-rich feed due to their high contents of non-fibre carbohydrates (Liu *et al.*, 2018) and, therefore, can replace cereal grains in livestock diets. This strategy is highly desirable because it reduces human food waste and alleviates the competition of livestock with crop food production. From a nutritional point of view, BP contain more sugars (mostly sucrose) and fat than starchy cereal grains (Humer *et al.*, 2018; Liu *et al.*, 2018). More recent research of our team has also shown that feeding of BP as replacement for cereal grains to dairy cows diversifies the energy nutrients of the cow's diet promoting nutrient intake and milk yield and giving rise to a numerical (non-significant) increase in milk fat concentration (Kaltenecker *et al.*, 2020). We have speculated that the higher milk energy production from feeding BP diets might be associated with more lipogenic precursors for mammary de novo synthesis of fatty acids.

Not only the content of dietary fat but also the shift in the pattern of the fatty acids related to BP feeding could considerably affect milk fatty acid composition. The latter aspect is of potential importance to consumer health because of the putative health-related effects of different fatty acids. Not many studies have investigated fatty acids of BP, but it is conceivable that the fatty acid profile changes dramatically compared to the native grains used for baking due to additions of dairy and plant oil components. For instance, Humer *et al.* (2018) showed that BP contain large amounts of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) dominated by 18:1 n9 as opposed to typical dairy feed ingredients which predominantly contain polyunsaturated fatty acids (PUFA). However, the fatty acid composition of ruminant lipids (milk, meat) does not directly reflect that of dietary sources because of the biohydrogenation of fatty acids in the rumen. In general, decreasing SFA and increasing PUFA proportions (especially of n3 origin) of edible products is considered favourable due to their potential health-beneficial effects (FAO, 1994; Simopoulos, 2002). The contribution of feeding BP to the nutritional quality of milk fat, however, has not been studied. As an extension of the previous research with BP (Kaltenecker *et al.*, 2020, 2021), the present study investigated whether different inclusion levels of BP also modify the fatty acid composition of the milk fat. A further aim was to assess the effect of the feeding period on the milk fatty acid composition, as well as potential interactions between diet and feeding period. We expected to see an increase in C18

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fatty acid especially of MUFA and mammary de novo fatty acid fractions in the milk fat of cows fed BP.

Materials and methods

Animals, diets and experimental design

The experimental design and feeding are reported in more detail in our companion studies (Kaltenegger *et al.*, 2020, 2021). The experimental procedures involving animal handling and treatment were approved by the institutional ethics committee of the University of Veterinary Medicine (Vetmeduni) Vienna and the national authority according to §26 of the Law for Animal Experiments, Tierversuchsgesetz 2012- TVG (GZ: 68.205/80-V/3b/2018).

Twenty-four Simmental cows in mid-lactation were used (mean \pm SD: days in milk of 149 ± 22.3 d, parity of 2.63 ± 1.38 , initial BW of 756 ± 89.6 kg and mean energy corrected milk yield of 30 ± 0.3 kg at the start of the experiment). All cows started with a baseline diet containing 50% forage and 50% concentrate (wheat and triticale as the main cereal grains) on a dry matter (DM) basis for 7 d. Then they were blocked by days in milk, parity, feed intake and milk yield and randomly assigned to one of the three test diets ($n = 8$ per diet) including CON (same as the baseline diet), 15% BP (a diet containing 15% BP in replacement of wheat and triticale) and 30%BP (30% BP and no cereal grains) and tested for 4 weeks. All test diets contained the same forage sources (grass silage and corn silage, 1 : 1 on DM basis) and forage to concentrate ratio of 50 : 50 (DM basis). The dried BP was milled, mixed, and pelleted together with the other ingredients of the concentrate. The diet was then prepared as a total mixed ration and fed to animals twice a day (07.30 and 15.00). Feed refusal was removed daily before filling in freshly mixed feed. Cows were fed individually ad libitum and the daily intake was recorded. Cows always had access to fresh clean water and salt blocks throughout the trial. Cows were milked twice a day at 07.00 and 17.30. For fatty acid analysis, the pooled morning and afternoon milk samples were taken from each cow at the end of baseline, day14, day21, and day28 during the test period. The milk samples were stored at -20°C until analysis. Details on diet ingredients, chemical composition, feeding management, and housing are presented in Kaltenegger *et al.* (2020).

Fatty acid analysis of diets and milk

The fatty acid content and composition of diets were analysed using a one-step extraction and methylation using 10% methanolic HCl under heat (90°C) according to Palmquist and Jenkins (2003). Heptadecanoic acid (17 : 0, H3500, Sigma-Aldrich, Saint Louis, MO) was used as an internal standard. For milk samples, frozen samples were thawed at room temperature shortly before analysis. The extraction and transesterification of fatty acids in the milk were done using a sodium methylate solution (5% w/v) (Suter *et al.*, 1997). Prior to reaction, a mixture of internal standards including glycerol trivalerate (93498, Sigma-Aldrich, Saint Louis, MO), tridecanoic acid, (T3882, Sigma-Aldrich, Saint Louis, MO), methyl undecanoate (94118, Sigma-Aldrich, Saint Louis, MO), and methyl nonadecanoate (74208, Sigma-Aldrich, Saint Louis, MO) was added. Fatty acid methyl esters (FAME) present in diets or milk samples were taken and analysed using a gas chromatograph (GC-2010 Plus, Shimadzu, Japan) equipped with a FID detector and a $100\text{ m} \times 0.25\text{ mm} \times 0.2\text{ }\mu\text{m}$ CP Sil-88 for

FAME (CP7489, Agilent Technologies, Santa Clara, CA). Peak identification of FAME was achieved by comparison with external standards including FAME mixture (Supelco 37 Component FAME Mix, Supelco, Bellefonte, PA), linoleic acid, conjugated methyl ester (O5632, Sigma-Aldrich, Saint Louis, MO), and Cis/Trans FAME Mix (35079, Restek, Bellefonte, PA). Fatty acids were quantified using the internal and external standards following the AOAC official method (AOAC, 2012).

Calculations and statistical analysis

Fatty acid intake was calculated from the analysed fatty acid composition and measured weekly average feed intake of individual cows. The compositions of fatty acids of milk samples are reported as relative concentrations (% of total fatty acids). $\Delta 9$ -Desaturase activity index, atherogenicity index (AI), thrombogenicity index (TI), and hypercholesterolaemic and hypocholesterolaemic ratio (Hh) were calculated following previous publications (Vessby *et al.*, 2013; Giuffrida-Mendoza *et al.*, 2015).

Data of fatty acid intake, milk fatty acid composition, and fatty acid indices (thrombogenicity index (TI), atherogenicity index (AI) and hypercholesterolaemic to hypocholesterolaemic ratio (Hh ratio) were analysed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The statistical model included fixed effects of diet, feeding time and their interaction and random effects of cow and block. We focused on the transitional change from baseline to test diets; baseline values were evaluated as an independent time point. Repeated measures within the cow were considered in the model and a spatial power covariance structure was used because the time points were unequally spaced. Data are reported as least-squares means and comparisons among diets or time of feeding were done following the Tukey's method when the corresponding fixed effect was found significant ($P \leq 0.05$).

Results and discussion

The fatty acid composition of the BP and complete diets is shown in Table 1. The dietary fatty acid composition of CON was dominated by $18 : 2\text{ n-6} > 18 : 1\text{ n9} > 16 : 0 > 18 : 3\text{ n3}$, accounting for 80% of total fatty acids. The increasing level of BP in replacement of cereal grains increased the proportion of $18 : 1\text{ n9}$ at the expense of $18 : 2\text{ n6}$ and $18 : 3\text{ n3}$. With 30% BP, $18 : 1\text{ n9}$ became the most abundant fatty acid accounting for almost one-third of fatty acids in the profile. Due to the high lipid content of BP, the absolute intake (g/d) of dietary fatty acids increased with increasing BP level in the diet (Table 1). Notably, the change was more evident for SFA and MUFA intake than for PUFA intake in which both BP groups resulted in similar PUFA intake. Cows in this study were in a positive energy balance (Kaltenegger *et al.*, 2020), thus diets were the major contributors to milk fat synthesis.

Diet affected the milk fatty acid composition and an interaction between diet and time was found on several fatty acids (Table 2) generally because of the swift changes from baseline to BP diets (online Supplementary Table S1). Following the shift of fatty acid intake with BP diets, the proportion of total $18 : 1$ fatty acids in milk fat increased (Table 2), which was maintained throughout the feeding period in 30% BP (Fig. 1a). The major milk fatty acid ($18 : 1$) was $18 : 1\text{ n9}$, which linearly increased with increasing BP level in the diet ($P < 0.001$, Table 2). With minimal body fat mobilization, as in the present

Table 1. Composition of major fatty acids in the diets (% of total fatty acids), dietary fatty acid content and the effect of dietary treatment on daily fatty acid intake

Item	Diet ^a				SEM	P value ^b	Linear ^c
	BP	CON	15%BP	30%BP			
Fatty acid composition (% of total fatty acid)							
12 : 0	3.18	0.24	1.14	1.75	–	–	–
14 : 0	3.12	0.48	1.11	1.64	–	–	–
16 : 0	18.08	16.34	15.87	16.84	–	–	–
18 : 0	4.11	2.02	2.85	3.47	–	–	–
18 : 1 n9	40.33	20.48	27.08	31.20	–	–	–
18 : 2 n6	21.22	33.01	28.78	25.40	–	–	–
18 : 3 n3	2.15	10.05	8.07	6.46	–	–	–
n6 : n3 ratio	9.88	3.30	3.57	3.94	–	–	–
SFA	32.68	28.73	29.78	31.05	–	–	–
MUFA	42.41	23.55	29.97	34.02	–	–	–
PUFA	23.41	43.53	37.11	32.01	–	–	–
Total fatty acids (% of DM)	8.78	2.10	2.78	3.59	–	–	–
Intake (g/d)							
16 : 0	–	77 ^a	98 ^b	129 ^c	5	<0.001	*
18 : 0	–	9.3 ^a	16.6 ^b	24.9 ^c	0.6	<0.001	*
18 : 1 n9	–	96 ^a	160 ^b	227 ^c	8	<0.001	*
18 : 2 n6	–	156 ^a	185 ^b	206 ^c	11	<0.001	*
18 : 3 n3	–	46 ^a	52 ^{a,b}	53 ^b	4	0.011	*
SFA	–	135 ^a	183 ^b	236 ^c	7	<0.001	*
MUFA	–	110 ^a	179 ^b	249 ^c	9	<0.001	*
PUFA	–	204 ^a	239 ^b	261 ^b	15	<0.001	*
Total fatty acids	–	451 ^a	600 ^b	747 ^c	29	<0.001	*

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Values sharing no common superscripts differ significantly ($P < 0.05$) according to Tukey's method.

^aCON diet contained, on DM basis, 30% grain (wheat and triticale) and 0% bakery by-products (BP) in the total diet, 15%BP diet contained 15% grains and 15% BP, and 30%BP diet contained 0% grains and 30% BP. All diets had, on DM basis, identical proportions of other main ingredients (25% grass silage, 25% corn silage, and 17% rapeseed meal) in the total diet.

^bThere were effects of week of sampling ($P < 0.001$) and its interaction with dietary treatment ($P < 0.001$, except for 18 : 3 n3 $P = 0.045$). For all fatty acids, the effect of dietary treatment on intake was detected after baseline and maintained through the trial.

^cContrast analysis: variables with a significant linear effect ($P < 0.01$) are marked with asterisk. No quadratic effect was detected.

study, this milk fatty acid originates mainly from diet and mammary desaturation of 18 : 0 (Chilliard *et al.*, 2000). Since the dietary supply of 18 : 1 n9 was plentiful and milk 18 : 0 was similar between both BP diets, it seems plausible that the diet was the predominant source driving the differences in the milk among treatments. Similar 18 : 1 n9-promoting effects have been reported with other 18 : 1 n9 rich feedstuffs such as olive by-products (Abbeddou *et al.*, 2011; Castellani *et al.*, 2017).

C18 PUFA 18 : 2 n6 and 18 : 3 n3 are essential fatty acids and the daily requirements can be met only by dietary sources. However, in contrast to the effect found with 18 : 1 n9, the increased intake of 18 : 2 n6 and 18 : 3 n3 *via* BP inclusion did not lead to their enrichment in the milk fat (Table 2), even when the daily supply amount of 18 : 2 n6 was as high as that of 18 : 1 n9 (Table 1). In ruminants, C18 unsaturated fatty acids are modified by ruminal microbes in the process called biohydrogenation. Their presence in ruminant lipids thus depends on their escape from ruminal biohydrogenation. Therefore, our data suggest substantial lipolysis of BP lipids and subsequent biohydrogenation of fatty acids in the rumen. The

higher transfer of 18 : 1 n9 into milk fat could be explained by its generally lower biohydrogenation rate in the rumen compared to C18 PUFA (Khiaosa-ard *et al.*, 2009).

Conjugated linoleic acids (CLA) along with numerous 18 : 1 isomers are intermediates of biohydrogenation of C18 unsaturated fatty acids in the rumen. In the mammary gland, CLA is largely synthesized endogenously from *trans*11 18 : 1 (Chilliard *et al.*, 2000). The naturally occurring CLA are considered to be potential health-beneficial fatty acids due to their multiple effects including anticarcinogenic, antiatherogenic, antidiabetogenic and immune-modulating properties (Rainer and Heiss, 2004). Interestingly, 30% BP favoured an enrichment of *trans*-18 : 1 (1.65 times the CON, $P < 0.05$) and CLA (1.59 times the CON, $P < 0.05$) in milk fat (Table 2). The positive effect on milk CLA was seen instantly when switching from baseline to 30% BP diet and was maintained throughout the trial (Fig. 1b). On the other hand, 15% BP did not improve over CON in this regard. It might be that the high BP diet led to surplus production of biohydrogenation intermediates, especially *trans*11 18 : 1, that could

Table 2. Composition of fatty acids in milk fat (% of total fatty acids), fatty acid secretion (kg/d) and fatty acid indices from cows fed diets without (CON, 8 cows) or with 15% or 30% bakery by-products (BP, 8 cows per each BP diet)

Item	Diet			SEM	P value			Linear ^a
	CON	15%BP	30%BP		Diet	Time ^b	Interaction	
4:0	2.53	2.46	2.72	0.25	0.509	0.715	0.314	NS
6:0	3.52	3.49	3.50	0.19	0.993	0.006	0.554	NS
8:0	1.97	2.04	1.96	0.11	0.797	0.501	0.117	NS
10:0	4.84	4.72	4.39	0.21	0.306	0.536	0.560	NS
12:0	5.90 ^a	5.57 ^{a,b}	5.10 ^b	0.21	0.030	0.099	0.760	*
iso 13:0	0.03	0.02	0.02	0.01	0.941	0.669	0.723	NS
anteiso 13:0	0.16 ^a	0.15 ^a	0.12 ^b	0.01	<0.001	0.831	0.596	*
14:0	15.39 ^a	15.12 ^{a,b}	14.37 ^b	0.29	0.041	0.279	0.249	*
14:1	1.32	1.33	1.04	0.10	0.036	0.511	0.208	*
15:0	2.31 ^a	1.88 ^{a,b}	1.66 ^b	0.17	0.021	<0.001	<0.001	*
iso 15:0	0.27	0.25	0.22	0.02	0.078	0.170	0.180	*
anteiso 15:0	0.59	0.54	0.52	0.03	0.213	0.414	0.328	NS
Iso 16:0	0.28	0.24	0.23	0.05	0.427	0.170	0.107	NS
16:0	31.66	30.38	29.41	0.76	0.066	<0.001	<0.001	*
16:1 n7	1.90	1.57	1.65	0.13	0.091	0.002	0.004	*
17:0	0.63 ^a	0.55 ^{a,b}	0.50 ^b	0.03	0.005	<0.001	<0.001	*
iso 17:0	0.43	0.46	0.43	0.03	0.604	0.169	0.063	NS
anteiso 17:0	0.69	0.69	0.67	0.03	0.835	0.092	0.775	NS
18:0	5.09 ^b	6.18 ^a	6.75 ^a	0.30	0.001	<0.001	0.010	*
18:1 n9 (cis 9) ^c	14.24 ^b	15.93 ^{a,b}	17.43 ^a	0.51	<0.001	<0.001	<0.001	*
18:1 other cis	1.30	1.25	1.19	0.08	0.200	0.001	0.199	NS
18:1 trans 10+11	0.83	0.93	1.17	0.12	0.117	0.018	0.351	*
18:1 other trans	0.46 ^b	0.68 ^a	0.97 ^c	0.05	<0.001	<0.001	<0.001	*
Sum 18:1 trans	1.30 ^b	1.62 ^b	2.15 ^a	0.16	<0.001	<0.001	0.011	*
Σ18:1	16.82 ^b	18.78 ^a	20.74 ^c	0.57	<0.001	<0.001	<0.001	*
CLA ^d	0.34 ^b	0.40 ^b	0.54 ^a	0.02	<0.001	<0.001	0.021	*
18:2 n6	1.52	1.42	1.54	0.11	0.491	0.624	0.004	NS
18:3 n3	0.33	0.30	0.32	0.03	0.582	0.194	0.436	NS
Other long-chain fatty acids	1.35	1.33	1.44	0.05	0.087	0.081	0.004	NS
De novo origin ^e	34.22	33.47	32.12	0.91	0.263	0.696	0.445	NS
OBCFA ^f	5.49 ^b	4.86 ^a	4.47 ^a	0.17	<0.001	<0.001	<0.001	*
SFA	74.05 ^a	72.59 ^{a,b}	70.59 ^b	0.72	0.005	<0.001	<0.001	*
MUFA	20.32 ^b	21.91 ^{a,b}	23.65 ^a	0.62	0.001	<0.001	<0.001	*
PUFA	2.90	2.89	3.24	0.18	0.111	<0.001	<0.001	NS
Fatty acid secretion	1.02 ^b	1.11 ^{a,b}	1.22 ^a	0.65	0.020	0.119	0.297	*
Indices ^g								
n6:n3	4.73	4.80	4.97	0.18	0.288	0.113	0.630	NS
Desaturase	2.81	2.59	2.60	0.11	0.307	0.001	0.132	NS
TI	4.31 ^a	4.04 ^{a,b}	3.71 ^b	0.12	0.004	<0.001	<0.001	*
AI	4.49 ^a	4.10 ^{a,b}	3.66 ^b	0.14	0.001	<0.001	<0.001	*

(Continued)

Table 2. (Continued.)

Item	Diet			SEM	P value			Linear ^a
	CON	15%BP	30%BP		Diet	Time ^b	Interaction	
Hh	2.94 ^a	2.61 ^{a,b}	2.33 ^b	0.10	<0.001	<0.001	<0.001	*

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

The feeding phase consisted of baseline and week 2, 3 and 4 of the test period.

Values sharing no common superscripts differ significantly ($P < 0.05$) according to Tukey's method.

^aContrast analysis: variables with a significant linear effect ($P < 0.05$) are marked with asterisk and NS for non-significance. No quadratic effect was detected.

^bIncluding 7-d baseline (all cows received the same diet as CON) and week 2, 3 and 4 of the respective test diet

^cCoelutions with 1–4% of minor isomers (*cis*6-8) in the total *cis*-isomer fraction

^d*Cis*-9, *trans*-11 conjugated linoleic acid as the major isomer

^e Σ 4:0–14:0

^fThe sum of odd- and branched chain fatty acids

^g $n6:n3 = \Sigma n6 / \Sigma n3$, Desaturase = $18:1\ n9 / 18:0$, TI (thrombogenicity index) = $(14:0 + 16:0 + 18:0) / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n6) + (3 \times \Sigma n3) + (\Sigma n3 / \Sigma n6)]$, AI (atherogenicity index) = $[12:0 + (4 \times 14:0) + 16:0] / (\Sigma n6 + \Sigma n3 + \Sigma MUFA\ n9)$, and Hh (hypercholesterolaemic to hypocholesterolaemic ratio) = $(14:0 + 16:0) / (18:1\ n9 + 18:2\ n6 + 20:4\ n6 + 18:3\ n3 + 20:5\ n3\ 22:5\ n3 + 22:6\ n3)$

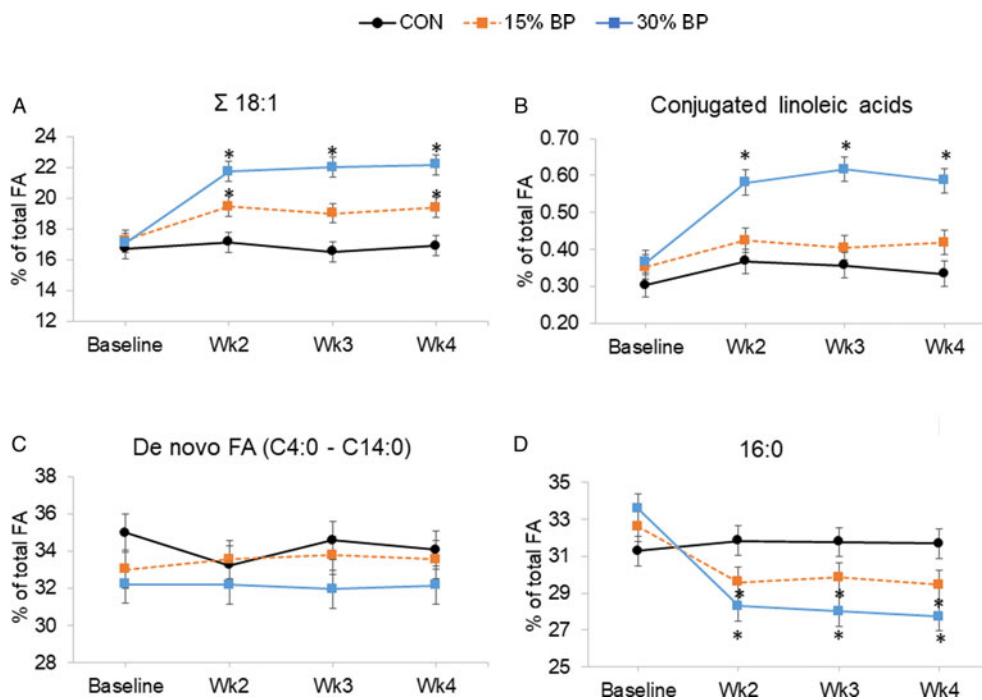


Fig. 1. Changes in percentages of selected fatty acids and groups of fatty acids in response to time of feeding of diets without (CON) or with 15 or 30% bakery by-products (BP). All cows received the same diet as CON in the baseline period. Except for fatty acids synthesized de novo (De novo FA), there are effects of diet, time and their interaction ($P \leq 0.01$). Within each diet, asterisks indicate significant changes compared to their baseline ($P < 0.05$) according to Tukey's method.

bypass the rumen. Odd- and branched-chain fatty acids in milk fat are largely derived from ruminal microbes (Vlaeminck *et al.*, 2006). The lower proportions of these fatty acids in the milk fat despite more fatty acids secreted in the milk with BP diets may indicate that the presence of BP affected the rumen microbial population. This assumption is challenged by the fact that ruminal pH was not negatively affected by BP inclusion (Kaltenegger *et al.*, 2020). Direct proof of the BP effect on ruminal microbiota in cows is currently not available, but a diet with 45% BP can impair ruminal fermentation and decrease microbial diversity as shown in vitro (Humer *et al.*, 2018). At the hindgut level, high BP inclusion could increase the odds for hindgut dysbiosis of animals (Kaltenegger *et al.*, 2021). Although 30% BP proved to be the most effective diet to enrich milk CLA, this high BP level might not be optimal for energy metabolism in mid-lactation cows (Kaltenegger *et al.*, 2020) and may negatively affect

the microbiota. Additional research in biohydrogenation of BP lipids would be necessary to find ways to promote bypass of such beneficial fatty acids to human edible products without the need for high inclusion levels of BP.

We showed previously that BP diets increased energy corrected milk yield (29.4, 31.5 and 34.3 kg/d, respectively) and caused a numerical increase in milk fat content (3.59%, 3.75% and 3.90% for CON, 15% BP and 30% BP, respectively), although neither this nor total fat yield achieved significance (Kaltenegger *et al.*, 2020). In the current analysis, we showed a linear increase in fatty acid secretion with increasing BP in the diet (Table 2). On the one hand, increased production of lipogenic precursors with dietary sugars (Oba *et al.*, 2015) may suggest more fatty acids synthesized de novo (4:0–14:0) in the mammary gland with BP diets. On the other hand, the provision of dietary lipids of long-chain origins (C18) could decrease mammary de novo

fatty acid synthesis (Chilliard *et al.*, 2000). However, we found that the total proportion of milk 4:0 to 14:0 was not disturbed by BP (Table 2, Fig. 1c). Thus, the increased milk energy production by BP diets reported earlier (Kaltenegger *et al.*, 2020) and more fatty acid secretion reported here was probably explained by greater uptake of the preformed fatty acids. It seems that the effect of long-chain fatty acids on reducing mammary de novo fatty acid synthesis could be compensated by a provision of carbohydrate sources that stimulate the production of lipogenic precursors (acetate and butyrate). Furthermore, with high-fat diets, there would be an increase in acetate needed for cholesterol biosynthesis (Liepa *et al.*, 1978) to accommodate the prioritized transportation of more lipid to the tissues and organs. With a low production of acetate, mammary de novo fatty acid synthesis might be substantially limited. In our study, a slight but significant decrease in 12:0 and 14:0 in milk fat with 30% BP compared to CON was already evident (Table 2). Therefore, a pairing of groups of energy nutrients is important for the production and secretion of milk fatty acids in dairy cows.

Among SFA, 16:0 was the most abundant and the most responsive SFA to dietary change (Table 2, Fig. 1d). As C18 MUFA and CLA proportions in milk fat increased, the 16:0 proportion dropped, which could be considered beneficial as it contributed to a decrease in the SFA proportions of milk fat. In agreement with our results, studies underlined that increasing dietary fat content decreases milk 16:0 percentages (Ungerfeld *et al.*, 2019) and percentages of C16 and C18 fatty acid families in milk are negatively correlated (Chilliard *et al.*, 2003). Due to the suggestion that dietary lipids may be linked to the incidence of coronary heart diseases, Ulbricht and Southgate (1991) suggested using fatty acid indices (TI and AI) of foods. The Hh ratio has also been referred to (Giuffrida-Mendoza *et al.*, 2015). These indices include 14:0 and 16:0 as the main SFA, with MUFA and n6 and n3 PUFA as the unsaturated counterparts in the estimations. Lower indices are desirable. In our study, 30% BP significantly reduced all indices compared to CON (Table 2) mainly because of the decrease in 16:0 at the expense of MUFA in milk fat. BP did not modify the proportions of milk PUFA, especially the n3 series, which otherwise would have been even more favourable. Omega-3 fatty acids are known for their effects on reducing the risk of many chronic diseases (Simopoulos, 2002). The basal diet used in the current study was based on maize silage, an n-6 PUFA source. Associative effects between BP lipids and n-3 PUFA rich feeds like pasture, grasses and hay remain to be addressed by future research.

In conclusion, the inclusion of BP in dairy rations increased milk fatty acid secretion, shifted the milk fatty acid profile to more 18:1 fatty acids at the expense of 16:0 and improved health indices of the milk fat while maintaining the mammary de novo synthesized fatty acids (4:0–14:0). A high inclusion rate of 30% BP offered an additional benefit by enriching CLA contents in milk fat. There was no enrichment of PUFA despite more intake of these fatty acids, suggesting substantial lipolysis and biohydrogenation of BP lipids in the rumen. These results indicate that the inclusion of BP as replacement for cereal grains increases the lipogenic property of the diet for milk fatty acid secretion. This alternative energy source has an added value in improving the nutritional quality of milk fat in relation to increased CLA and reduction of SFA, both effects being potentially health-beneficial.

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