J. Agr. Sci. Tech. (2018) Vol. 20: 1383-1394

Pumpkin (*Cucurbita maxima* D.) Silage as a Feed that Improves Nutritional Properties of Cow's Milk

G. Halik[1*], A. Lozicki [1], J. Wilczak [2], E. Arkuszewska [1], and M. Makarski [1]

ABSTRACT

The study was performed with 40 Simmental cows divided into four groups (treatments). The group I received diet with grass silage, maize silage, and concentrate. Group II was fed similar as group I plus 400 mg d^{-1} cow^{-1} of *β*-carotene. In group III, 40% maize silage DM was replaced with pumpkin silage to ensure a 400 mg higher *β*-carotene intake compared to the group I. In group IV, 60% of maize silage DM was replaced with pumpkin silage without balancing *β*-carotene. Milk samples were collected at 4 and 8 weeks of experiment. The milk was analyzed for the basic composition, carotenoids, Total Antioxidant Status (TAS), and composition of fatty acids. In both measurements, the highest content of *α*-carotene, *β*-carotene, lutein, violaxanthin were in the milk of group IV, and the lowest in group I. In the milk groups III and IV, higher content of PUFA, including n-3 PUFA, was found in successive samplings. In all samplings, milk TAS in group I was significantly lower compared to groups III and IV.

Keywords: *β*-carotene, Carotenoids, Fatty acid, PUFA, Simmental cows.

INTRODUCTION

Milk and milk products are an important part of human diet. Fortification of milk with valuable bioactive compounds makes them more available for consumption by consumers. Cow's milk provides humans with large amounts of protein of high biological value, including antioxidant and antibacterial proteins, valuable fatty acids, but also bioactive compounds such as carotenoids and minerals (Butler *et al*., 2008; Kuczyńska *et al*., 2013). Cow productivity as well as the nutritional and dietetic values of milk are largely determined by feeding. The proper selection of feeds and balancing of rations allows for improving the milk yield and modifying the chemical composition of milk (Brodziak *et al*., 2012). During the summer period, the dietetic value of milk can be improved by feeding the cows with high forage rations (Slots *et al*., 2009). This improvement is more difficult to achieve when cow diets are based on conserved feeds, namely, silages. Maize silage, which is most frequently fed to cows, is poor in compounds such as carotenoids (Alves *et al*., 2011). In the case of wilted grass silage or lucerne silage, valuable bioactive components are lost during wilting (Dunne *et al*., 2009). One feed that can be a valuable source of bioactive compounds, such as carotenoids, is pumpkin silage. This feed seems a good solution for organic farms, where animal nutrition is primarily based on natural feeds. Pumpkin silage has a high content of carotenoids, including *β*-carotene and other bioactive compounds, such as flavonoids

[1]Department of Animal Nutrition and Biotechnology, Faculty of Animal Science, Warsaw University of Life Sciences, Ciszewskiego 8, 02-786 Warsaw, Poland.
[2] Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland.
*Corresponding author; e-mail: gabrysiahalik@wp.pl

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

(Łozicki *et al*., 2015). Due to its content of seeds, this feed has an interesting composition of fatty acids (Łozicki *et al*., 2015). The content of these components in pumpkin silage is much higher than in grass silage or maize silage (Alves *et al*., 2011, Łozicki *et al*., 2015).

The carotenoids, flavonoids, and valuable polyunsaturated fatty acids present in pumpkin silage can have a positive influence on the health, antioxidant potential, and reproductive performance of cows (Michal *et al*., 1994; Arechiga *et al*., 1998; Spears and Weiss, 2008). The increased dietary intake of valuable bioactive compounds by the cows should also translate into their higher content in milk, thus improving its nutritive and dietetic value.

The aim of the study was to analyze the effect of a feed ration containing pumpkin silage on the nutritive and dietetic value of milk and, consequently, on the functional properties of milk.

MATERIALS AND METHODS

Animals

The study was conducted with 40 Simmental dairy cows divided into 4 groups (treatments). The experimental animals were selected based on the analogue principle according to age, lactation, milk production, and body weight. The cows were enrolled in the experiment 4 weeks prior to the expected calving date. During the experiments, cows were individually housed in a tie-stall barn and had free access to water. At the beginning, they received diets with tested feeds for dry cows and, after calving, the diets for lactating cows. Feed ration was the variable differentiating the experimental groups. In group I, standard diet based on grass silage and maize silage was used. Group II received an increased dose of *β*-carotene in the form of 400 mg of synthetic *β*-carotene cow^{-1} d^{-1}. The rations for cows in experimental groups III and IV were supplemented with pumpkin silage. In group III, part of maize silage was replaced with pumpkin silage to ensure a 400 mg higher intake of natural *β*-carotene compared to group I. This corresponded to replacing 40% DM of maize silage with pumpkin silage. Rations in group IV were not balanced for *β*-carotene content, while 60% DM of maize silage was replaced with pumpkin silage.

Diets were formulated according to INRA (2009) and prepared as TMR. The ingredients and chemical composition of the diets are shown in Table 1. TMR was prepared for the whole groups in order to supply 105-110% of the expected feed intake for the group. Cows were individually fed twice daily (50% of total diet in each feeding) at 0700 and 1700 hours. The feed refusals for the group were collected daily and weighted once a day prior to the morning feeding. The feed intake for the group was recorded daily as the difference between feed offered and refused. The average feed intake per cow was calculated by dividing the feed intake per group by the number of animals.

Samples (0.5 kg) of diets and orts from each group were taken once weekly before and during the morning feeding during the experimental period. Composite samples were taken and stored at −20°C for further chemical analysis.

Samples

Cows were milked twice daily (0600 and 1600 hours). During the collection period (at 4 and 8 weeks of lactation), the milk of each milking was weighed and sampled. Samples were refrigerated after the morning milking and mixed with samples of the afternoon milking.

After sampling, fresh milk was subjected to some chemical analyses. Part of the milk was frozen at -20°C until the laboratory analyses. Basic milk composition, carotenoid content, fatty acid profile, and Total Antioxidant Status (TAS) were determined.

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

Table 1. Composition, nutritive value, and dry matter intake of the diets.

Feeds and nutrients	Group I	Group II	Group III	Group IV
		(DM g kg^{-1})		
Grass silage	380	380	390	400
Maize silage	300	300	190	150
Pumpkin silage	-	-	120	170
Brewer's grains	40	40	40	40
Concentrate	280	280	260	240
Synthetic β-carotene	-	+	-	-
Nutritive value of 1 kg DM of the diets				
UFL [a]	1.01	1.01	1.05	1.06
PDIN (g) [b]	103.41	103.41	104.25	103.78
PDIE(g) [c]	93.64	93.64	95.48	95.61
Crude protein(g)	170.19	170.19	173.31	173.92
NDF(g) [d]	388.68	388.68	390.01	395.40
Crude ash(g)	41.87	41.87	47.01	50.08
β-Carotene (mg)	66.03	83.71	84.42	92.60
Crude fat(g)	39.11	39.11	40.51	41.55
Fatty acid composition [a] (g 100 g^{-1} of total FA)				
C12:0	0.31	0.31	0.28	0.27
C14:0	0.32	0.32	0.33	0.32
C15:0	0.15	0.15	0.15	0.15
C16:0	16.73	16.37	16.49	16.37
C16:1c9	1.07	1.07	1.01	0.99
C17:0	0.15	0.15	0.14	0.13
C18:0	1.73	1.73	1.97	2.06
C18:1c9	22.61	22.61	22.88	22.68
C18:2n-6	26.82	26.82	27.87	28.29
C20:0	0.44	0.44	0.42	0.42
C18:3n-3	24.7	24.7	24.6	24.9
C22:0	0.49	0.49	0.47	0.47
C24:0	0.48	0.48	0.48	0.49
SFA [e]	20.81	20.81	20.98	21.05
MUFA [f]	25.18	25.18	24.77	24.04
PUFA [g]	51.56	51.56	52.47	53.51
Dry matter intake (kg d^{-1})	20.3	20.5	20.0	20.6

[a] The feed unit milk production; [b] Protein Digested in the Intestines of the Nitrogen; [c] Protein Digested in the Intestines of Energy; [d] Neutral Detergent Fiber; [e] Saturated Fatty Acid; [f] Mono-Unsaturated Fatty Acid, [g] PolyUnsaturated Fatty Acid;

Chemical Analysis

The chemical composition of the feeds was determined according to AOAC (2005): dry matter by drying at 105°C to constant weight, crude ash by incineration at 550□C for 6 hours; crude protein (N×6.25) by using the micro-Kjeldahl technique (Kjeltec System 1026 Distilling Unit, Foss Tecator, Sweden); and crude fat after extraction with petroleum ether by the Soxhlet method. Neutral Detergent Fiber (NDF) in feed was determined according to Van Soest *et al*. (1991). The NDF was expressed as the ash-free residue after extraction with boiling neutral solutions of sodium lauryl sulfate and EDTA in a Tecator apparatus.

Basic chemical composition of milk (fat, crude protein, lactose, solids, non-fat solids) and urea was determined by

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

automated infrared analysis with a MilkoScan FT 120 apparatus (Foss Electric). The yield of Energy Corrected Milk (ECM) was calculated by the following formula:

ECM= ([0.327×kg milk]+[12.97×kg fat]+[7.2×kg protein])

For fatty acids profile analysis, lipids were extracted according to the method by Folch *et al*. (1957) and fatty acids were esterified following the standard AOAC method (2005). The fatty acids analysis was conducted using a TRACE GC ULTRA gas chromatograph (Thermo Electron Corporation) on a SUPELCOWAX^{TM} 10 Capillary GC Column (30 m×0.25 mm×0.25 μm) under the following conditions of the separation process: Carrier gas – helium at a flow rate of 7.5 mL min^{-1}, injector temperature – 220^{o}C, column temperature – 190^{o}C 3 minutes - 3^{o}C min^{-1} -220^{o}C-35 minutes, and detector temperature – 250^{o}C.

The analysis of carotenoid separation and contents was made by applying the HPLC system (Dionex) equipped with a CoulArray electrochemical detector (ESA Inc). The separation was conducted on a Hypersil BDS 150□4.6 mm, 5 μm column (Sigma-Aldrich) at a mobile phase flow rate of 1.2 mL min^{-1}. The mobile phase consisted of a methanol:isopropanol mixture (98:2). The conditions of electrochemical detection were four electrodes with potentials 400, 500, 600, and 750 mV. The chromatograms were processed by identifying the pigments on the basis of standards and areas of chromatographic peaks, taking into account their retention times as well as the ratio of the peak area for the dominating electrode to that of neighboring electrodes.

Total Antioxidant Status (TAS) was determined by the Randox Test. The assay was based on spectrophotometric measurement of the degree of color change of the reactive radical ABTS® (2-2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) during the time specified by the Randox application.

Statistical Analysis

Results obtained were analyzed statistically using the ANOVA procedure of one-way analysis of variance with Statgraphics 6.0 Plus software. The significance of differences between groups was identified using F-test. The analysis involved the effect of feeding on the specified parameters. Results were presented in tables as mean values of parameters, standard errors of the means, and statistical significance of the effect.

RESULTS

Production and Composition of Milk

The first measurement of cow milk production and composition revealed that milk yield was lowest in group I and highest in group IV ($P \leq 0.05$) (Table 2). Feed ration had no effect on the composition of milk. In the second measurement, milk yield was the highest in group IV, with significant differences in relation to groups I ($P \leq 0.01$), II and III ($P \leq 0.05$). Differences were found in milk composition. In groups I and II, fat contents were significantly higher than in groups III ($P \leq 0.05$) and IV ($P \leq 0.01$). Protein content was the highest in group IV and the lowest in group I ($P \leq 0.01$). Also, lactose content was the highest in group IV, with highly significant difference in relation to groups I and II ($P \leq 0.05$).

Fatty Acid Composition of Milk

During both measurements, no differences in total SFA content were established between the groups. At the first sampling, among saturated fatty acids, the highest C4:0 content was found in group IV and the lowest in group I ($P \leq 0.05$) (Table 3). The

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

Table 2. Effect of feed ration on milk yield and composition.[a]

Item	Experimental group				SE	P-value
	I	II	III	IV		
First measurement: 4 weeks after calving						
Milk production (kg)	20.71b	23.08	24.38	26.55a	1.584	0.048
Milk ECM (kg)	23.27b	25.63	27.70	29.71a	1.657	0.051
Fat (%)	4.36	4.21	4.35	4.29	0.153	0.521
Protein (%)	3.18	3.30	3.38	3.37	0.078	0.210
Lactose (%)	4.82	4.78	4.74	4.90	0.077	0.489
Urea (mg L^{-1})	203.04	187.00	208.28	223.42	14.299	0.365
Second measurement: 8 weeks after calving						
Milk production (kg)	21.81 B	23.16b	24.28b	28.06Aa	1.285	0.007
Milk ECM (kg)	24.75b	26.56b	26.94b	31.18a	1.440	0.024
Fat (%)	4.42Aa	4.52Aa	4.15b	4.09B	0.130	0.035
Protein (%)	3.23B	3.33	3.37	3.51A	0.070	0.001
Lactose (%)	4.66b	4.68b	4.84	4.95a	0.088	0.043
Urea (mg L^{-1})	213.51	203.28	202.71	191.42	16.140	0.801

[a] Values in the same row marked as AB, CD differ at $P \leq 0.01$; ab, cd at $P \leq 0.05$; Milk ECM: Energy Corrected Milk.

Table 3. Effect of feed ration on the fatty acid composition of milk: First measurement.[a]

Item	Group				SE	P-value
	I	II	III	IV		
	g 100 g^{-1} FAME (Fatty Acid Methyl Esters)					
C4:0	3.37 b	3.58 ab	3.55ab	3.89 a	0.128	0.049
C6:0	2.53	2.63	2.64	2.59	0.073	0.735
C8:0	1.68	1.65	1.68	1.48	0.075	0.178
C16:0	29.37 Bb	28.18 B	32.91 A	31.83Aa	0.854	0.002
C17:0	0.46	0.464	0.41	0.42	0.023	0.266
C18:0	8.98 B	11.07 A	8.61B	9.29 B	0.431	0.002
C20:0	0.08 AB	0.09 A	0.06 B	0.06 B	0.006	0.003
SFA	67.87	67.78	69.26	67.28	1.646	0.137
C10:1	0.25 a	0.217 ab	0.22 ab	0.18 b	0.016	0.050
C14:1	0.65 a	0.56 ab	0.56ab	0.52 b	0.033	0.042
C16:1 n-9	0.54 BD	0.59 B	0.64 C	0.72 A	0.023	0.001
C16:1 n-7	1.77	1.49	1.54	1.71	0.099	0.169
C17:1	0.28	0.22	0.19	0.24	0.025	0.123
C18:1n-9	23.60	24.47	19.68	24.07	0.524	0.122
C18:1n-7	2.37	2.12	2.11	2.06	0.121	0.288
C20:1	0.04 a	0.04 a	0.03 b	0.04 a	0.002	0.035
MUFA	29.53	29.72	25.01	29.56 ab	1.599	0.126
C18:2n-6	1.73 b	1.76 b	1.98 a	2.03 a	0.068	0.021
C18:3n-6	0.06 AB	0.07 A	0.05 B	0.05 B	0.003	0.005
C18:3n-3	0.26 BD	0.28B	0.30 BC	0.33A	0.012	0.001
CLA	0.41	0.40	0.38	0.36	0.026	0.467
PUFA	2.50 b	2.48 b	2.73 ab	2.77 a	0.086	0.049
n-6	2.24 ab	2.20 b	2.42 a	2.43 a	0.077	0.050
n-3	0.26 BD	0.28 B	0.30 BC	0.33 A	0.012	0.001
n6/n3	8.65 B	7.93 A	8.02 B	7.31 A	0.225	0.001

[a] Values in the same row marked as AB, CD differ at $P \leq 0.01$; ab, cd at $P \leq 0.05$.

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

milk of cows from groups III and IV had the highest content of C16:0, with a significant difference in relation to groups I and II (P≤ 0.01). The highest proportions of C18:0 – significantly higher than in groups I, III and IV (P≤ 0.01) and of C20:0 were observed in group II. Among MUFA, C10:1 and C14:1 had the highest proportion in group I and the lowest in group IV (P≤ 0.05). The milk of cows from group IV had significantly more C16:1n-9 compared to group III (P≤ 0.05) and groups I and II (P≤ 0.01). Total MUFA content was similar in all the groups. The proportion of C18:2 n-6 in the milk of groups III and IV was significantly higher than in groups I and II (P≤0.05). Group II, in relation to groups III and IV, showed a significantly higher proportion of C18:3 n-6. The highest proportion of C18:3 n-3 was noted in group IV, with a highly significant difference in relation to groups I and II (P≤ 0.01). The proportion of this acid in groups I and II was also significantly lower than in group III (P≤ 0.05). The total PUFA content was highest in group IV, significantly exceeding the content observed in groups I and II (P≤ 0.05). In groups III and IV, in relation to group II, there was a much higher content of n-6 PUFA. The proportion of n-3 PUFA in group IV was remarkably higher than in groups I and II (P≤ 0.01), and that in group III was significantly higher in relation to group I (P≤ 0.05). The n6/n3 ratio was lowest in group IV and highest in group I (P≤ 0.01).

At the second sampling (Table 4), among SFA, the highest proportion of C16:0 was present in groups III and IV, with a big difference in relation to groups I and II (P≤ 0.01). C17:0 was most abundant in milk of group II and least abundant in groups III and IV (P≤ 0.01). The proportion of this acid in group I was also significantly higher than in group IV (P≤ 0.01). In group II, compared to the other groups, there was the highest proportion of C18:0 (P≤ 0.05). The proportion of C20:0 in groups I and II was much higher compared to groups III and IV (P≤ 0.01). The total SFA content was similar in all groups. The milk of group IV cows, compared to the other groups, contained significantly more C16:1 n-9. Also, the milk of cows from group III had a remarkably higher content of C16:1 n-9 in relation to groups I and II (P≤ 0.01). The proportion of C17:1 in the milk of groups I and II was significantly higher than in groups III and IV. The proportion of C18:1 n-9 was highest in milk of group II, with a clear difference in relation to groups III and IV (P≤ 0.05). No differences were found between the groups in total MUFA content. The proportion of C18:2 n-6 and C18:3 n-3 in the milk of group IV was significantly higher compared to groups I and II (P≤ 0.05). In groups I and II, in relation to group IV, there was a higher CLA content (P≤ 0.05). No differences were found between the groups in the total PUFA content. Group IV, compared to groups I and II, had a much higher content of n-3 PUFA (P≤ 0.05).

Milk Carotenoid Content and Total Antioxidant Status (TAS)

At the first measurement, the milk of groups III and IV was found to contain more α-carotene compared to groups I and II (P≤ 0.05) (Table 5). β-carotene was highest in the milk of group IV, with a significant difference in relation to group I (P≤ 0.05). The milk of cows from group IV contained more violaxanthin compared to groups I and II (P≤ 0.01) and III (P≤ 0.05), and more lutein compared to groups I and II (P≤ 0.05). Violaxanthin was also more abundant, compared to groups I and II, in the milk of group III (P≤ 0.05). The highest TAS was characteristic of the milk from group IV, with a statistically significant difference in relation to group I (P≤ 0.05).

Milk from the second measurement contained significantly more α-carotene in groups III and IV compared to groups I and II (P≤ 0.01) (Table 5). β-carotene content in the milk of group IV was significantly higher than in group I (P≤ 0.05), while lutein and violaxanthin content in groups III and IV was significantly higher compared to

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

Table 4. Effect of feed ration on the fatty acid composition of milk: Second measurement.[a]

Item	Group				SE	P-value
	I	II	III	IV		
	g 100 g^{-1} FAME (Fatty Acid Methyl Esters)					
C4:0	3.44	3.57	3.62	3.79	0.123	0.276
C6:0	2.53	2.56	2.56	2.68	0.069	0.416
C8:0	1.65	1.58	1.56	1.57	0.069	0.820
C16:0	29.59 A	28.55 A	33.77 B	33.59 B	0.809	0.001
C17:0	0.45 BC	0.49 C	0.41 AB	0.38 A	0.023	0.009
C18:0	8.97 a	10.32 b	8.81 a	9.09 a	0.421	0.049
C20:0	0.08 B	0.08 B	0.06 A	0.06 A	0.005	0.010
SFA	67.48	66.93	70.93	70.99	1.520	0.129
C10:1	0.25	0.22	0.21	0.22	0.016	0.497
C14:1	0.66	0.61	0.57	0.57	0.036	0.317
C16:1 n-9	0.59 A	0.60 AB	0.65 B	0.75 C	0.027	0.001
C16:1 n-7	1.83	1.48	1.59	1.60	0.099	0.102
C17:1	0.29 a	0.24 ab	0.21 a	0.19 a	0.024	0.044
C18:1n-9	24.14 ab	25.20 b	20.89 a	21.02a	1.439	0.041
C18:1n-7	2.19	2.19	2.11	2.03	0.102	0.059
C20:1	0.04	0.04	0.03	0.04	0.003	0.187
MUFA	29.98	30.60	26.31	26.44	1.528	0.104
C18:2n-6	1.75 b	1.71 b	1.88ab	2.06 a	0.067	0.021
C18:3n-6	0.06a	0.06a	0.05b	0.05b	0.003	0.050
C18:3n-3	0.25b	0.26b	0.28ab	0.29a	0.011	0.038
CLA	0.43 b	0.40 b	0.38 ab	0.30 a	0.031	0.041
PUFA	2.50	2.44	2.53	2.73	0.088	0.126
n-6	2.52	2.17	2.43	2.24	0.079	0.139
n-3	0.25b	0.26b	0.28ab	0.29a	0.011	0.045
n6/n3	8.76	8.32	8.17	8.00	0.218	0.107

[a] Values in the same row marked as AB, CD differ at $P \leq 0.01$; ab, cd at $P \leq 0.05$.

Table 5. Effect of feed ration on milk carotenoid content and Total Antioxidant Status (TAS).[a]

Item	Group				SE	P-value
	I	II	III	IV		
First measurement: 4 weeks after calving						
α-Carotene (ng mL^{-1})	222.74b	242.93b	330.43a	342.48a	69.811	0.050
β-Carotene (ng mL^{-1})	386.48b	435.30ab	470.34ab	523.34a	77.408	0.036
Lutein (ng mL^{-1})	64.93b	60.31b	73.50ab	83.69a	12.353	0.045
Violaxanthin (ng mL^{-1})	25.78Bb	27.00Bb	32.62ad	47.64Ac	4.695	0.009
Zeaxanthin (ng mL^{-1})	23.61	23.04	28.02	28.56	2.361	0.238
TAS (umol Trolox)	4.23b	4.87ab	5.12ab	5.26a	0.339	0.049
Second measurement: 8 weeks after calving						
α-Carotene (ng mL^{-1})	242.75B	256.99B	390.9A	401.72A	47.460	0.031
β-Carotene (ng mL^{-1})	393.06b	461.49ab	478.92ab	516.51a	82.474	0.042
Lutein (ng mL^{-1})	61.82b	60.40 b	87.82a	88.17a	14.629	0.041
Violaxanthin (ng mL^{-1})	23.44b	23.25b	35.62a	37.25a	5.007	0.032
Zeaxanthin (ng mL^{-1})	24.45	26.44	29.20	28.39	2.400	0.122
TAS (umol Trolox)	2.17Bb	3.87a	4.24 A	4.13 A	0.356	0.005

[a] Values in the same row marked as AB, CD differ at $P \leq 0.01$; ab, cd at $P \leq 0.05$.

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

groups I and II (P≤ 0.05). The milk from group II (P≤ 0.05) and groups III and IV (P≤ 0.01) also had a significantly higher TAS in relation to the milk from group I.

In all measurements, no significant differences were observed in *β*-carotene content between group II (supplemented with synthetic *β*-carotene) and the groups receiving pumpkin silage. There were also no significant differences between these groups in the TAS of milk.

DISCUSSION

Production and Basic Composition of Milk

During successive measurements, the highest milk production was noted in group IV (Table 2), in which cows were fed diets with the highest proportion of pumpkin silage. This may indicate that the cows from this group were more efficient in nutrient utilization. The rations for all the groups had similar energy and protein value. Also, the dry matter intake was similar in all groups (Table 1). What distinguished group IV was the greater proportion of *β*-carotene and other carotenoids in ration of dry matter. It can be assumed that this factor had an effect on the ruminal fermentation process. A study by Hino *et al*. (1993) shows that higher ruminal concentration of *β*-carotene and *α*-tocopherol has a positive influence on the activity and development of rumen microflora, including cellulolytic bacteria. Because cows from group IV consumed the highest dietary intake of *β*-carotene, this likely contributed to the higher activity of bacteria and better nutrient utilization. Antone *et a*l. (2015), who supplemented diets with *β*-carotene rich carrot, also reported improvements in cow yields and milk fat and protein content. The positive effect of a ration richer in *β*-carotene on productivity was also observed by Lotthammer (1979). However, the increase in dietary *β*-carotene is not always translated into increased milk production or improved chemical composition of milk. One example is a study by de Ondarza *et al*. (2009), who found that a diet supplemented with 400 mg of *β*-carotene had no impact on milk yield or milk fat content.

Analysis of the basic composition of milk showed that fat content was highest in groups I and II, and lowest in groups III and IV. Changes in fat content and fat composition are largely dependent on the concentration, composition and form of dietary fiber (crude fiber, ADF, NDF), as well as on the starch and sacharose content (Kuczyńska *et al*., 2011). The content of crude fiber and NDF was similar in all the rations, whereas the proportion of structural fiber was lower in groups III and IV. This could have affected the ruminal fermentation process by shifting fermentation towards acetic acid, which translated into a decrease in milk fat content.

The better nutrient utilization by animals fed with the diet high in carotenoids, also had an effect on milk protein and lactose content. The higher protein content was apparent in the groups receiving pumpkin silage, with a significantly higher protein content in group IV in relation to group I at the second measurement. Lactose content at the second sampling in group IV was significantly higher than in the other groups.

The protein content of milk is influenced by the dietary energy level and the content of non-structural carbohydrates. In the present study, the rations were formulated based on basic chemical composition and were characterized by similar energy and protein value. It can be assumed, however, that the higher intake of *β*-carotene and the greater proportion of pectins in pumpkin silage were the factors affecting ruminal metabolism and the availability of dietary energy, thus improving the energy value of the diet (Ben-Ghedalia *et al*., 1989, Hino *et al*., 1993).

Composition of Milk Fatty Acids

Cow's milk contains valuable fatty acids essential in human nutrition, such as butyric,

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

arachidic, CLA, and PUFA (Gabryszczuk *et al*. 2013). Unfortunately, undesirable saturated fatty acids (lauric and myristic) are also present in milk.

In the current study, successive measurements showed the highest content of butyric acid, valuable from the consumer's perspective, in the milk of group IV cows. In the first measurement (Table 3), this figure was significantly higher than in group I. Butyric acid has a positive effect on intestinal mucosa development and function, and is helpful in intestinal diseases (Banasiewicz *et al*., 2010). The higher content of this acid in the milk of group IV cows, which received the highest amount of pumpkin silage, may be due to the highest pectin content of the diet, which contributes to a shift in ruminal fermentation towards butyric acid. Research did not establish the effect of ration on the total SFA content of milk, including the undesirable lauric and myristic acids, which have hypocholesterolemic effects (Willet, 2012).

The use of pumpkin silage in the diets increased total PUFA content in milk. This was particularly noticeable in group IV, where PUFA was significantly more abundant at the second measurement compared to groups I and II (Table 4). The milk of cows from group IV, compared to groups I and II, was characterized during successive measurements by a higher content of C 18:3 n3, C 18:2 n6 and total n-3 acids, which are beneficial for human health. In group III, in which the proportion of pumpkin silage was lower than in group IV, the proportion of these acids was also higher than in groups I and II, but not all the measurements showed significant differences. The results obtained are probably due to the higher proportion of PUFA in the dry matter ration in groups III and IV (Table 1). Pumpkin, including pumpkin silage, is rich in palmitic, stearic, oleic and linoleic acids (Murkovic *et al*., 1996, Łozicki *et al*., 2015). Compared to maize silage, pumpkin silage has a higher PUFA and a lower MUFA content (Alves *et al*. 2011, Łozicki *et al*., 2015). Therefore, replacing part of maize silage with pumpkin silage could contribute to changes in the composition of milk fatty acids.

Apart from the better composition of fatty acids in pumpkin silage compared to maize silage, the factor that could contribute to increased PUFA content in the groups fed with pumpkin silage, could be the introduction of fat in the form of ground seeds. This factor could have limited the ruminal biohydrogenation of PUFA.

This may also be indicated by the lower CLA content of milk in groups III and IV compared to groups I and II, which was significantly lower at the second measurement in group IV in relation to groups I and II. Milk CLA content is influenced by ruminal biohydrogenation of linoleic acid (Schreiner and Windisch, 2006). A higher proportion of PUFA in milk when feeding seeds compared to oil was reported, among others, by Gulati *et al*. (2002).When analyzing the effect of pumpkin fed to cows on the composition of milk fatty acids, we found that similar results to ours were obtained by Kuczyńska *et al*. (2013), who fed cows with fresh pumpkin.

Milk Carotenoid Content and TAS

The present study demonstrated the effect of pumpkin silage on the carotenoid content of milk. During successive measurements, the content of the majority of the studied carotenoids was higher in groups III and IV compared to group I (control), and even group II (Table 5). A particularly marked increase in the milk carotenoid content was evident in group IV, which received the highest amount of pumpkin silage in the diet. The results obtained clearly show that higher dietary intake of carotenoids translates into their higher content in milk. Similar findings were reported for cows receiving other feeds rich in carotenoids (Antone *et al*., 2011; Antone *et al*., 2015).

Groups II and III were characterized by similar intake of β-carotene, but it differed in origin. In group II, 400 mg of β-carotene was synthetic, and in group III all of it was natural. β-carotene source had no effect on its content in milk. Comparison of milk β-carotene content in these two groups did not show

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

differences between them. Many studies reported better *β*-carotene availability from natural compared to synthetic sources (Melton, 2006; Baldi *et al*., 2008). Our results fail to confirm this. However, the use of pumpkin silage increased the content of other carotenoids in milk. Nielsen *et al*. (2004) showed that a higher proportion of maize silage in cow rations is one of the main factors responsible for the lower content of vitamins and antioxidants in the milk.

The higher carotenoid content in the milk of cows receiving pumpkin silage translated into a higher Total Antioxidant Status (TAS) of milk. This was particularly noticeable in the milk of group IV cows, which was characterized by higher TAS in relation to the control group during all measurements. Also the milk of group III cows showed higher total antioxidant status compared to the control group, which was confirmed by the second and third measurement. The high milk carotenoid content in cows fed maize silage and the high total antioxidant status of this milk are indicative of its high nutritive and dietetic value.

CONCLUSIONS

The study showed that pumpkin silage added to dairy cow rations improves the dietetic parameters of milk by increasing the carotenoid content of milk and the total antioxidant status of milk, and contributes to a higher proportion of PUFA (including n-3 PUFA) in milk. Pumpkin silage could be a valuable component of cow diets in organic farms or in those oriented towards producing milk with special nutritional properties. Synthetic and natural *β*-carotene added to the diets had no effect on its content in milk.

ACKNOWLEDGEMENTS

This study was supported by the Polish National Research Council grants: NCN NN311521740. The paper is a part of the PhD thesis of MSc Gabrieli Halik.

REFERENCES

1. Alves, S. P., Cabrita, R. J., Jerónimo, E., Bessa, R. J. B. and Fonseca, A. J. M. 2011. Effect of Ensiling and Silage Additives on Fatty Acid Composition of Ryegrass and Corn Experimental Silages. *J. Anim. Sci.,* **89:** 2537-2545.
2. Antone, U., Zagorska, J., Sterna, V., Jemeljanovs, A., Berzins, A. and Ikauniece, D. 2015. Effects of Dairy Cow Diet Supplementation with Carrots on Milk Composition, Concentration of Cow Blood Serum Carotenes, and Butter Oil Fat-Soluble Antioxidative Substances. *Agron. Res.,* **13(4):** 879–891.
3. Antone, U., Šterna, V. and Zagorska, J. 2011. The Influence of Cow Feed Enriched with Carrots on Milk Quality and Nutritional Value. *Proceedings of 17th Annual International Scientific Conference Research for Rural Development 2011*, University of Agriculture, Jelgava, Latvia, **1:** 80–86.
4. AOAC. 2005. *Official Methods of Analysis*. The Association of Official Analytical Chemists, AOAC Intl., Gaithersburg, USA.
5. Aréchiga C. F., Staples C. R., McDowell L. R. and Hansen P. J. 1998. Effects of Timed Insemination and Supplemental *β*-Carotene on Reproduction and Milk Yield of Dairy Cows under Heat Stress. *J. Dairy Sci*., **81:** 390-402.
6. Baldi, A., Cheli, F., Pinotti, L. and Pecorini, C. 2008. Nutrition in Mammary Gland Health and Lactation: Advances over Eight Biology of Lactation in Farm Animals Meetings. *J. Anim. Sci.,* **86:** 3–9.
7. Banasiewicz, T., Borycka-Kiciak, K., Kiciak, A., Kotunia, A., Zabielski, R. and Krokowicz, P. 2010. Butyric Acid in Bowel Inflammations. *Gastroenterol. Rev.,* **5(5):** 251–257.
8. Ben-Ghedalia, D., Yosef, E., Miron, J. and Est, Y. 1989. The Effects of Starch- and Pectin-Rich Diets on Quantitative Aspects of Digestion in Sheep. *Anim. Feed Sci. Technol.,* **24:** 289-298.
9. Brodziak, A., Litwińczuk, A., Topyła, B. and Wolanciuk, A. 2012. Influence of Production Season x Breed and Feeding System Interactions on Daily Yield and Milk Physical-Chemical Composition. *Sci. Ann. Polish Soc. Anim. Prod*., **8(1):** 19-27.

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

10. Butler, G., Nielso, J. H., Slots, T., Seal, Ch., Eyre, M. D., Sanderson, R. and Leifert, C. 2008. Fatty Acid and Fat-Soluble Antioxidant Concentrations in Milk from High and Low Input Conventional and Organic Systems: Seasonal Variation. *J. Sci. Food Agri.*, 88,1431–1441.
11. de Ondarza, M. B., Wilson, J. W. and Engstrom, M. 2009. Effect of Supplemental *β*-Carotene on Yield of Milk and Milk Components and on Reproduction of Dairy Cows. *Prof. Anim. Sci.*, **25:** 510-516.
12. Dunne P. G., Monahan F. J., O'Mara F. P., Moloney A. P. 2009. Colour of Bovine Subcutaneous Adipose Tissue: A Review of Contributory Factors, Associations with Carcass and Meat Quality and Its Potential Utility in Authentication of Dietary History. *Meat Science*, **81**: 28-45.
13. Folch, J., Lees, M. and Sloane-Stanley, G. H. 1957. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J. Biol. Chem.*, **226**: 497-509.
14. Gabryszuk, M., Sakowski, T., Metera, E., Kuczyńska, B. and Rembiałkowska, E. 2013. Effect of Feeding on Content of Bioactive Substances in Milk from Cows Raised in Organic Farms. *Food Sci. Technol. Quality*, **3(88):** 16 – 26.
15. Gulati, S. K, May, C., Wynn, P. C. and Scott, T. W. 2002. Milk Fat Enriched in n−3 Fatty Acids. *Anim. Feed Sci. Technol.*, **98**: 143–152.
16. Hino, T., Andoh, N. and Ohgi, H. 1993. Effect of *β*-Carotene and *α*-Tocopherol on Rumen Bacteria in the Utilization of Long Chain Fatty Acids and Cellulose. *J. Dairy Sci.*, **76:** 600-605.
17. IZ PIB-INRA.2009. Standards for Ruminant Nutrition. National Research Institute of Animal Production, Kraków.
18. Kuczyńska, B., Nałęcz-Tarwacka, T., Puppel, K., Gołębiewski, M., Grodzki, H. and Slósarz, J. 2011. The Content of Bioactive Components in Milk Depending on Cow Feeding Model in Certified Ecological Farms. *J. Res. Appl. Agric. Eng.*, **56(4).**
19. Kuczyńska, B., Nałęcz-Tarwacka, T. and Puppel, K. 2013. Bioactive Components as an Indicator of the Health-Beneficial Quality of the Milk. *Family Med.*, **1:** 11-18.
20. Lotthammer, K. H. 1979. Importance of *β*-Carotene for the Fertility of Dairy Cattle. *Feedstuffs*, **51**: 6.
21. Łozicki, A., Koziorzebska, A., Halik, G., Dymnicka, M., Arkuszewska, E., Niemiec, T. and Bogdan, J. 2015. Effect of Ensiling Pumpkin (*Cucurbita maxima* D.) with Dried Sugar Beet Pulp on the Content of Bioactive Compounds in Silage and Its Antioxidant Potential. *Anim. Feed Sci. Technol.*, **206:** 108-113.
22. Melton, L. 2006. The Antioxidant Myth: A Medical Fairy Tale. *New Sci. Mag.*, **5:** 40-43.
23. Michal, J. J., Heirman, L. R., Wong, T. S., Chew, B. P., Frigg, M. and Volker, L. 1994. Modulatory Effects of Dietary *β*-Carotene on Blood and Mammary Leukocyte Function in Periparturient Dairy Cows. *J. Dairy Sci.*, **77:** 1408-1421.
24. Murkovic, M., Hillebrand, A., Winkler, J., Leitner, E. and Pfannhauser, W. 1996. Variability of Fatty Acid Content in Pumpkin Seeds (Cucurbita pepo L.). *Eur. Food Res. Technol.*, **203(3):** 216–219.
25. Nielsen, J.H., Lund-Nielsen, T. and Skibsted, L. 2004. Higher Antioxidant Content in Organic Milk than in Conventional Milk Due to Feeding Strategy. *DARCOFenews, Newsletter from Danish Research Centre for Organic Farming*, **9(3).**
26. Schreiner, M. and Windisch, W. 2006. Supplementation of Cow Diet with Rapeseed and Carrots: Influence on Fatty Acid Composition and Carotene Content of the Butter Fat. *J. Food Lipids.*
27. Slots, T., Butler, G., Leifert, C., Kristensen, T., Skibsted, L. H. and Nielsen, J. H. 2009. Potentials to Differentiate Milk Composition by Different Feeding Strategies. *J. Dairy Sci.*, **92:** 2057–2066.
28. Spears, J. W. and Weiss, W. P. 2008. Role of Antioxidants and Trace Rlements in Health and Immunity of Transition Dairy Cows. *Vet. J.*, **176:** 70-76.
29. Van Soest P. J., Robertson J. B., Lewis B. A. 1991. Methods for Dietary Fiber,Neutral Detergent Fiber and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.*,**74**: 3583-3597.
30. Willet, W. C. 2012. Dietary Fats and Coronary Heart Disease. *J. Inter. Med.*, **272:** 13-24.

[DOR: 20.1001.1.16807073.2018.20.7.8.2]

سیلوی کدو حلوایی به عنوان یک علوفه خوراکی خواص غذایی شیر گاو را بهبود می بخشد

گ. هالیک، ا. لوزیکی، ج. ویلکزاک، ا. آرکوزوسکا، و م. ماکارسکی

چکیده

این پژوهش با ۴۰ گاو سیمنتال (Simmental) که در ۴ گروه (تیمار) دسته بندی شده بود اجرا شد. جیره غذایی گروه ۱ علف سبز سیلویی و کنسانتره بود. غذای گروه ۲ شبیه گروه۱ باضافه 400 میلی گرم بتا کارو تین(β-carotene) در روز برای هر گاو بود. در گروه ۳، ۴۰٪ از ماده خشک ذرت سیلویی با کدو حلوایی سیلویی جایگزین شد تا اطمینان حاصل شود که گاو ها ۴۰۰ میلی گرم بتا کاروتین بیشتر از گروه ۱ خواهند خورد. در گروه ۴، ۶۰٪ ذرت سیلویی با کدو حلوایی سیلویی جایگزین شد بدون اینکه بتا کاروتین تراز شود. در هفته های چهارم و هشتم بعد از شروع آزمایش، شیر گاو ها نمونه برداری شد. تجزیه شیرها برای تعیین ترکیب اصلی، کاروتین ها، وضعیت کل آنتی اکسیدان (TAS)، و ترکیب اسید های چرب انجام شد. در هر دو اندازه گیری، بیشترین میزان موجود آلفا کاروتین، luteinو violaxanthinدر شیر گروه ۴ و کمترین آن در گروه ۱ بود. در نمونه برداری های متوالی، در شیر گروه های ۳ و ۴ مقدار بیشتری PUFA ، از جمله n-3 PUFA، به دست آمد. در همه نمونه برداری ها مقدارTAS در شیر گروه ۱ به طور معناداری کمتر از گروه ۳ و ۴ بود.

[DOR: 20.1001.1.16807073.2018.20.7.8.2]