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The concentrations of β-carotene, vitamin A and vitamin E in bovine milk in regard to the feeding season and the share of concentrate in the feed ration

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The objective of the present study was to determine the β-carotene, vitamin A and vitamin E content of cow's milk in regard to the selected nutritional factors and the stage of lactation. The experimental materials consisted of milk samples from 90 Holstein-Friesian cows fed a partly mixed ration (PMR – maize silage, grass silage and concentrated feed). The contents of vitamin A, vitamin E and β-carotene in milk were determined primarily by the feeding season. A gradual decrease in vitamin concentrations in milk fat was observed during winter feeding. The inclusion of pasture sward into the feed ration for dairy cows caused an increase ($P \le 0.01$) in the concentrations of vitamin A, vitamin E and β-carotene in milk. A higher concentrate share in the diet (accompanied by a higher milk yield) determined also an increase in the vitamin A and vitamin E content of milk, but it had no effect on β-carotene levels. The desirable changes in the concentrations of vitamin A and vitamin E, observed along with an increase in the content of concentrate the diet, indicate that their levels in the ration should be optimized.

KEY WORDS: cows / milk / β-carotene / vitamin A / vitamin E

Milk is an important and well-recognized source of fat-soluble vitamins (especially vitamin A and E) in the diet,. In addition milk contains small amounts of β-carotene. Above-mentioned components exert antioxidant activity, they delay the oxidation of milk fat and therefore they protect polyunsaturated fatty acids, which are susceptible to oxidation reactions. Moreover, they counteract undesirable changes such as changes of taste and smell of milk and its products. Additionally, they exert antitumor activity through the capture of free radicals [7, 12]. They also play an important role in the immune response and prevention of mastitis [4]. Concentration of vitamin A and E and β-carotene in milk is determined by various factors, including feeding season, management conditions, lactation stage, herd performance, health of mammary gland and genetic factors [1, 6, 10]. The research results indicate that milk from cows fed extensively and grazing on ecological pastures is characterized by the highest content of biologically active ingredients, including vitamins. The lowest seasonal variations in content of functional components of milk are recorded under feeding with total mixed ration (TMR) in ad libitum conditions throughout the year [11, 13]. It is noteworthy that information concerning the transfer of lipophilic vitamins from the feed through the blood and then to the mammary gland (milk) is still limited.

The present study was designed to analyze the content of β-carotene and vitamins A and E in the milk of cows with regard to certain nutritional factors (feeding period, the level of feeding concentrate), and the lactation stage.

Materials and methods

The study was conducted on 90 cows of Polish Holstein-Friesian breed of Black and White variety in 1-4 lactation. The mean yield of milk per lactation was 6900 kg milk. Cows were managed in the free stall barns with access to the feed bunk. The study included two periods of cows feeding:

- alcove nutrition (from November to April) – group 1,
- summer nutrition (from May to June) – group 2.

Nutrition was applied in the PMR system with the use of a feed wagon. The PMR dose consisted of conserved fodder (corn silage, grass silage), the breeder mash and a protein supplement (containing extracted soybean crushed meal protected from degradation in the rumen). During the summer nutrition, cows from group 2 were grazing on the pasture for a few hours, in addition to the feeding with silages and the breeder mash. The share of concentrate in the feeding ration was differentiated regarding the milk yield of cows (group 1 and 2). Cows with a daily milk yield extending 22 kg of milk received individual addition of 0.5 kg of breeder mash for every kilogram of milk produced additionally. An automatic feeding station and animal identification system were used in the process.

Primary dose of PMR, developed by INRA standards [5], supplied about 20 kg dry matter. The concentration of nutrients in 1 kg of dry matter of the dose (in winter) was as following: LFU – 0.96; crude protein – 13.7%; PDI – 97 g; raw fibre – 20.4%; NDF – 36.7%; ADF – 21.4%; Ca – 0.84%; P – 0.41%; Mg – 0.21%; β-carotene – 31 mg; vitamin E – 17 mg; vitamin A – 2146 IU.

Milk samples for analysis were collected from cows 3 three times (in period from November to April) and in June (after 6 weeks of pasture grazing.) The milk samples were examined to determine the fat content (Milko-Scan apparatus 133B, Foss Electric, Denmark), the level of β-carotene by means of Manz and Bühler-Steinbrunn method [8], the vitamin A and E concentrations were investigated according to Hewavitharan et al [3] separation method using HPLC apparatus with prior protein removal by addition of ammonia, ethanol and n-hexane.

The obtained results were analyzed statistically using computer program Statistica 8.0 Pl.

Results and discussion

A systematic decrease in concentration of β-carotene in milk fat (group 1) was observed during the winter season of feeding. In January, the content of this component was lower

Table 1 – Tabela 1
The β-carotene, vitamin A and vitamin E content of cow's milk in successive months of feeding
Zawartość β-karotenu, witaminy A i E w mleku krów w poszczególnych miesiącach żywienia

Specification Wyszczególnienie		November Listopad	January Styczeń	March Marzec	June Czerwiec
β-carotene β-karoten					
μg/g milk	$\overline{x}$	0,080^{A}	0,076^{A}	0,065^{A}	0,137^{B}
μg/g mleka	Sd	0,021	0,026	0,017	0,031
μg/g of fat	$\overline{x}$	1,821^{A}	1,777^{A}	1,622^{A}	3,160^{B}
μg/g tłuszczu	Sd	0,524	0,479	0,504	0,714
Vitamin A Witamina A					
μg/g milk	$\overline{x}$	0,315^{ABC}	0,376^{C}	0,277^{A}	0,347^{BC}
μg/g mleka	Sd	0,072	0,081	0,069	0,088
μg/g of fat	$\overline{x}$	7,220^{AB}	8,826^{C}	6,925^{A}	8,020^{BC}
μg/g tłuszczu	Sd	1,289	1,478	1,596	1,471
Vitamin E Witamina E					
μg/g milk	$\overline{x}$	0,452^{A}	0,482^{A}	0,367^{A}	1,566^{B}
μg/g mleka	Sd	0,081	0,094	0,078	0,479
μg/g of fat	$\overline{x}$	10,341^{A}	11,321^{A}	9,167^{A}	36,170^{B}
μg/g tłuszczu	Sd	2,024	2,137	1,946	5,783

$\overline{x}$ – mean – średnia; Sd – standard deviation – odchylenie standardowe
A, B, C– values in the rows with different letters differ significantly (P≤0.01) – P≤0,01

by 5% (0.076 μg/g), and in March by 18.5% (0.065 μg/g of milk) compared with the values obtained in milk samples analyzed in November (0.080 μg/g) – Table 1.

The contents of vitamins A and E in milk were the highest in January and the values clearly decreased in milk samples collected in March (respectively by 26% and 24%). It should be noted that the levels of vitamins (especially vitamin E) in milk from cows fed PMR dose in winter were relatively low (from 10.341 μg/g fat in November to 36.170 μg/g fat in June), despite of the full-filled nutritional requirements of animals. Jensen et al [6] emphasize that only a small amount of tocopherols and retinol ester from the feed is transferred into milk. Yeargan et al [17] suggest that the secretion of vitamin E into milk is often below the physiological level of – 45 μg/g of milk fat.

The share of pasture in the feeding ration of cows (group 2) resulted in 4 times higher content of vitamin E in milk, 2 times increased levels of β-carotene and 25% more vitamin A as compared with values obtained in milk samples collected in March (P≤0.01). The increase of vitamin A, E and β-carotene concentrations in milk fat obtained from grazing period corresponds with results obtained by other authors [7, 15]. The above-mentioned increase is derived from the feeding with fresh green pasture, which is characterized by a higher concentration of β-carotene and tocopherols [9, 10, 14]. The authors emphasize that grazing on the pasture caused an increase in the concentration of vitamins in milk regardless of the production system. Research performed by Reklewska et al [13] showed

Table 2 – Tabela 2
The concentrations of β-carotene, vitamin A and vitamin E in cow's milk as dependent on the concentrate content of the ration
Zawartość β-karotenu, witaminy A i E w mleku krów w zależności od udziału paszy treściwej w diecie

Specification Wyszczególnienie		Proportion of concentrates in the diet Udział paszy treściwej w diecie		
		32%	15%	8,1%
β-carotene β-karoten				
µg/g milk	$\overline{x}$	0,075	0,070	0,079
µg/g mleka	Sd	0,020	0,025	0,021
µg/g of fat	$\overline{x}$	1,913	1,636	1,857
µg/g tłuszczu	Sd	0,523	0,594	0,481
Vitamin A Witamina A				
µg/g milk	$\overline{x}$	0,344	0,321	0,311
µg/g mleka	Sd	0,067	0,093	0,067
µg/g of fat	$\overline{x}$	8,721	7,521	7,240
µg/g tłuszczu	Sd	1,698	2,163	1,569
Vitamin E Witamina E				
µg/g milk	$\overline{x}$	0,453	0,429	0,419
µg/g mleka	Sd	0,087	0,096	0,077
µg/g of fat	$\overline{x}$	11,490	10,000	9,778
µg/g tłuszczu	Sd	2,211	2,242	1,797

$\overline{x}$ – mean – średnia; Sd – standard deviation – odchylenie standardowe

that the milk from cows grazing on the pasture contained more vitamins (0.32 vs. 0.26 mg/l) than milk from cows fed with TMR diet. Yearlong studies carried out by Ellis et al [2] confirm that grazing on the pasture and type of preserved forage used in the ration have an impact on the concentration of vitamins A and E and β-carotene in milk. A high share of silage in the feeding ration of cows may lead to a reduction of the concentration of discussed vitamins in milk, as compared with a dose containing fresh fodder. Conserved fodder may have an impact on reducing the β-carotene level [10], which is the main precursor of vitamin A in ruminants [9].

The results of the present study showed that increased concentrate share in the feed ration of cows had an influence on the increase (approximately 17.5%) of vitamin E concentrations in milk (9.778 vs. 1.490 µg/g fat) – Table 2. A higher share of concentrate in the feeding ration of cows (coinciding with an increase in milk yield), determined the increase of the vitamin A content in milk (from 7.240 µg/g fat at 8.1% share of the concentrate to 8.721 µg/g fat, when the animals received a diet that consisted of approximately 32% of concentrate in the dry matter). It is noteworthy that a breeder mash was the only source of vitamin A in the feeding ration of cows. Nevertheless, the share of concentrate in feed in the diet did not have any impact on the β-carotene content in milk (1.636-1.913 µg/g fat).

Table 3 – Tabela 3

The β-carotene, vitamin A and vitamin E content of cow's milk during successive stages of lactation
Zawartość β-karotenu, witaminy A i E w mleku krów w poszczególnych okresach laktacji

Specification Wyszczególnienie	Months Miesiąc	Days of lactation Dni laktacji					
		≤60		61 – 120		121 – 180	
		$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
β-carotene β-karoten							
µg/g milk µg/g mleka	November – March listopad – marzec	0,072	0,027	0,071	0,020	0,079	0,021
µg/g of fat µg/g tłuszczu		1,816	0,683	1,669	0,467	1,862	0,482
µg/g milk µg/g mleka	June czerwiec	0,118	0,028	0,145	0,037	0,130	0,014
µg/g of fat µg/g tłuszczu		2,730	0,653	3,342	0,864	3,002	0,316
Vitamin A Witamina A							
µg/g milk µg/g mleka	November – March listopad – marzec	0,333	0,068	0,314	0,072	0,322	0,093
µg/g of fat µg/g tłuszczu		8,359	1,610	7,373	1,765	7,539	2,169
µg/g milk µg/g mleka	June czerwiec	0,409	0,065	0,335	0,056	0,319	0,045
µg/g of fat µg/g tłuszczu		9,453	1,480	7,734	1,293	7,367	1,051
Vitamin E Witamina E							
µg/g milk µg/g mleka	November – March listopad – marzec	0,420	0,083	0,455	0,103	0,419	0,077
µg/g of fat µg/g tłszczu		10,557	2,088	10,683	2,420	9,801	1,801
µg/g milk µg/g mleka	June czerwiec	1,546	0,245	1,533	0,415	1,723	0,461
µg/g of fat µg/g tłuszczu		34,704	5,649	35,409	8,815	39,787	9,651

It is well-established that the concentrates contain relatively low levels of β-carotene [10]. The results obtained by Ellis et al [2] have shown that increased share of concentrate in the feed ration of cows (the conventional system of production), coincided with an increase of vitamin A content in milk, without significant alterations in the vitamin E and β-carotene concentrations. The authors emphasize that many prominent dairy farms apply concentrates supplemented with additions of synthetic vitamins, which can affect their concentration in milk. Weiss and Wyatt [16] studied effects of the increase of vitamin A concentration (from 25 to 125 and 250 IU in 1 kg of dry matter of the dose) in the feeding ration of cows, they found a significant increase ($P \leq 0.01$) in the concentration of vitamin E (respectively from 0.44 mg to 0.74 mg and 1.16 mg per liter of milk). Results of the present research indicate certain changes in the vitamins concentration in milk from cows in different lactation stages. During the winter feeding, the lowest concentrations of β-carotene and vitamin A in milk were observed between the 61st and 120th day after birth, and for vitamin E the lowest values were observed between 121st and 180th day of lactation (Table 3). The study performed by Jensen et al [6] showed that the content of vitamin E was the lowest in the second week of lactation (13.3 μg/g fat) and then it gradually increased, reaching 36.7 μg/g fat at the end of lactation. The vitamin A concentration in the first week of lactation was 11.3 μg/g fat, after 5 weeks of lactation the value decreased to 5.6 μg/g fat, and then increased again to 13.8 μg/g fat. In the present study, the share of pasture in feeding ration determined a gradual increase in the vitamin E concentration as the lactation progressed. During this period of feeding, the content of vitamin A in milk was higher in the first 60 days after birth, and the highest concentration of β-carotene was found between the 61st and 120th day of lactation. Nevertheless, the obtained differences were not statistically significant. According to Calderon et al [1], the secretion of β-carotene as well as secretion of vitamins A and E into milk is variable and depending on the lactation period only to a limited extent. According to Noziére et al [11], the energy status of cows has a major impact on the secretion rate i.e. the low-energy intake is accompanied by a reduction of concentration of the vitamin A and E and β-carotene in milk. According to Mc Dowell [9], an increased additional requirement for vitamins (especially vitamin E) occurs in lactating cows receiving feeding dose with a high concentration of polyunsaturated fatty acids (PUFA), and also in conditions of stress or a deficiency of selenium in the diet.

The conducted study showed that:

– concentrations of vitamin A and E and β-carotene in the milk are determined mainly by the feeding period of cows. The inclusion of grass forage (a natural source of β-carotene and tocopherols) to the diet resulted in an increase ($P \leq 0.01$) of these components in the milk;

– raising share of concentrate in the feed ration of cows (coinciding with a higher milk yield) increased the level of vitamin A and E in the milk, however it did not stimulate the increase of the β-carotene concentration;

– more attention should be paid not only to the feeding possibilities of increasing milk yield, but also the possibility of increasing the level of lipophilic vitamins and other antioxidants in milk fat.

REFERENCES

1. CALDER'ON F., CHAUVEAU-DURIOT B., MARTIN B., GRAULET B., DOREAU M., NOZIÉRE P., 2007 – Variations in carotenoids, vitamins A and E and color in cow's plasma and milk during late pregnancy and the first three months of lactation. ***Journal of Dairy Science*** 90, 2335-2346.
2. ELLIS K.A., MONTEIRO A., INNICENT G.T., GROVE-WHITE D., CRIPPS P., MCLEAN W.G., HOWARD C.V., MIHM M., 2007 – Investigation of the vitamins A and E and β caro-

tene content in milk from UK organic and conventional dairy farms. ***Journal of Dairy Research*** 74, 484-491.
3. HEWAVITHARANA A.K., VAN BRAKEL A.S., HARNETT M., 1996 – Simultaneous liquid chromatographic determination of vitamins A, E and β carotene in common dairy foods. ***International Dairy Journal*** 6, 613-624.
4. HOGAN J.S., WEISS W.P., SMITH K.L., SORDILLO L.M., WILLIAMS S.N., 1996 – α-Tocopherol concentrations in milk and plasma during elinical Escherichia coli mastitis. ***Journal of Dairy Science*** 79, 71-75.
5. INRA, 2001 – Normy żywienia Bydła, Owiec i Kóz . Instytut Zootechniki, Kraków.
6. JENSEN S.K., JOHANNSEN A.K.B., HERMANSEN J.E., 1999 – Quantitative secretion and maximal secretion capacity of retinol, β carotene and α-tocopherol into cows' milk. ***Journal of Dairy Research*** 66, 511-522.
7. LINDMARK-MÁNSSON H., AKESSON B., 2000 – Antioxidative factors in milk. ***British Journal of Nutrition*** 84, 103-107.
8. MANZ U., BŰHLER-STEINBRUNN I.I., 1987 – Analytical methods for vitamins and carotenoids in feed (edited by H.E. Keller). Department of Vitamin Research and Development, Roche, Bazylea.
9. MC DOWELL L.R., 2000 – Vitamins in Animal and Human Nutrition. Second Edn. Ames IW, USA: Lowa State University Press.
10. NOZIÉRE P., GRAULET B., LUCAS A., MARTIN B., GROLIER P., DOREAU M., 2006 – Carotenoids for ruminants: from forages to dairy products. ***Animal Feed Science and Technology*** 131, 418-450.
11. NOZIÉRE P., GROLIER P., DURAND D., FERLAY A., PRADEL P., MARTIN B., 2006 – Variations in carotenoids, fat-soluble micronutrients and color in cows' plasma and milk following changes in forage and feeding level. ***Journal of Dairy Science*** 89, 2634-2648.
12. PARODI R.W., 1999 – Conjugated linoleic acid and other anticarcinogenic agents of bovine milk. ***Journal of Dairy Science*** 82, 1339-1349.
13. REKLEWSKA B., BERNATOWICZ E., REKLEWSKI Z., NAŁĘCZ-TARWACKA T., KUCZYŃSKA B., ZDZIARSKI K., OPRZĄDEK A., 2003 – Zawartość biologicznie aktywnych składników w mleku krów zależnie od systemu żywienia i sezonu. ***Zeszyty Naukowe Przeglądu Hodowlanego*** 68, z. 1, 85-98.
14. SHINGFIELD K.J., SALO-VÄÄNÄNEN P., PAHKALA E., TOIVONEN V., JAAKKOLA S., PIIRONEN V., HUHTANEN P., 2005 – Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. ***Journal of Dairy Research*** 72, 349-361.
15. TOLEDO P., ANDRÉN A., 2003 – Content of β carotene in organic milk. ***Food, Agriculture and Environment*** 12, 122-125.
16. WEISS W.P., WYATT D.J., 2003 – Effect of dietary fat and vitamin E on α-tocopherol in milk from dairy cows. ***Journal of Dairy Science*** 86, 3582-3591.
17. YEARGAN M.R., OSHIDARI S., MITCHELL G.E., TUCKER R.E., SCHELLING G.T., HEMKEN R.W., 1979 – Mammary transfer of vitamin E in cows treated with vitamin A or linoleic acid. ***Journal of Dairy Science*** 62, 1734-1738.

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Zawartość β-karotenu oraz witaminy A i E w mleku krów w zależności od okresu żywienia i udziału paszy treściwej w dawce pokarmowej

S t r e s z c z e n i e

Badania miały na celu analizę koncentracji β-karotenu oraz witaminy A i E w mleku krów, z uwzględnieniem niektórych czynników żywieniowych (okres żywienia, poziom żywienia paszą treściwą) oraz okresu laktacji. Doświadczeniem objęto 90 krów rasy holsztyńsko-fryzyjskiej żywionych systemem PMR (kiszonka z kukurydzy, sianokiszonka z traw oraz mieszanka treściwa). Zawartość β-karotenu oraz witaminy A i E w mleku była uwarunkowana okresem żywienia krów. W czasie żywienia zimowego obserwowano systematyczny spadek poziomu badanych witamin. Włączenie runi pastwiskowej do dawki pokarmowej krów zwiększało ($P \leq 0,01$) zawartość β-karotenu i witaminy A i E w mleku. Wzrastający udział paszy treściwej w dawce pokarmowej krów (wraz z wyższą wydajnością mleczną) wpłynął na wzrost poziomu witaminy A i E w mleku, nie stymulował natomiast wzrostu koncentracji β-karotenu. Obserwowane tendencje korzystnych, z punktu widzenia dietetyki, zmian w koncentracji witaminy A i E w miarę zwiększania żywienia krów paszą treściwą, wskazują na potrzebę optymalizacji ich poziomu w mieszance paszowej.

SŁOWA KLUCZOWE: krowy / mleko / β-karoten / witamina A / witamina E