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Influence of lactation stage on selected blood parameters and biological value of cow milk during pasture season in organic system of production

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Abstract: Influence of lactation stage on selected blood parameters and biological value of cow milk during pasture season in organic system of production. The disproportion between the cow's genetically determined ability to produce milk and the limits to improving the energy value of feed may be the cause of the metabolic stress of animals kept at organic farms. Negative energy balance has a major impact on the body's hormonal balance and the function of the organs; it also affects the content of basic components (protein, fat and lactose) in cows' milk. The aim of the study was to investigate changes in the biological value of organic milk obtained from high producing dairy cows at different stages of lactation during pasture season. The study was carried out on 68 Polish Holstein-Friesian primiparous. 272 milk samples were collected from cows which were in one of the following phases: the beginning of lactation (BL): 5-30 days post partum; peak of lactation (PL): 60-90 days post partum; full lactation (FL): 120-150 days post partum; end/final phase of lactation (EL): 250 days post partum. Stage of lactation had an overriding effect on the majority of analyzed milk constituents including: whey proteins, vitamins soluble in fat, phospholipids and fatty acids. The highest levels of lactoferin were found at the beginning of lactation (BL). At the same time, the β-hydroxybutyrate (BHBA) concentration increased, which confirmed that the fast growing milk yield at the peak of lactation is linked to an

increase in energy demand. The lowest alanine aminotransferase and non esterified fatty acids activity was measured during BL. The study has shown a significant influence of lactation stage on the biological value of organic milk obtained from high producing dairy cows during pasture season. In addition, there is evidence that the organic production system has no influence on the metabolic profile of cows as the plasma parameters were within their standard norms. This might be the result of the positive effect of grass antioxidants.

Key words: cow's milk, lactation stage, organic system

INTRODUCTION

Recent studies have pointed out significant differences in the chemical composition and nutritional quality of milk and dairy products between organic and conventional farming systems (Bisig et al. 2007). Previous studies stated that the feeding regime had a major effect on the FA profiles of milk (Dewhurst et al. 2006, Elgersma et al. 2006) but that other factors (including breed, genotype or both, the stage and number of lactations) may also influence milk composition (Walker

et al. 2004, Chilliard et al. 2007). One of the biggest issues in organic herds is to provide high yielding cows with sufficient amounts of nutrients to meet their needs while maintaining environmental standards for the composition of the ratio and type of feed (Hermansen 2003). In conventional farms negative energy balance usually appears at the beginning of lactation (Ingvartsen et al. 2003) and is associated with a simultaneous rapid increase in the milk productivity of animals and limited capacity for feed intake. Due to seasonal changes in the composition and quality of the feed cows from organic farms might also suffer an energy deficit in other stages of lactation. A more direct insight into the physiological state of the cow's organism is provided by the concentration of certain metabolites in the blood plasma, milk or both. It was reported that the level of β-hydroxybutyric acid (BHBA) presented an accurate picture of the energy balance in grazing dairy cows. Moreover, there was a direct link between an excess of free radicals in cows' organisms and decreased disease resistance. There are many components in plants that might peroxidation prevent lipid through limitation of free radicals, stimulation of antioxidant enzymes activity (i.e.: superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) or both. These properties strongly depend upon the presence of phenolic compounds in plants such as flavonoids, tannins, phenolic acids and terpenes, phenols as well as certain vitamins (i.e. E, C and A). Of all plant components the flavonoids, also called bioflavonoids, are the most powerful antioxidants. They have the ability to inhibit the enzymes responsible

for the production of superoxide anions (i.e. xanthine oxidase, protein kinase C), as well as the chelation of transition metals; "cleaning up" free radicals and initiating and stimulating antioxidant protection of other factors. Flavonoids are predominantly found in legumes and especially grassland legumes such as clovers (Steinshamn et al. 2008).

There is a direct relationship between the availability of excess free radicals and a decrease in animal disease resistance. Therefore, increasing the level of antioxidants in the diet of cows through pasturing is a natural way to stimulate the immune system of cows in organic production systems. The nutritional value of organic pastures is mainly associated with herbage variety (different species of grasses, legumes and herbs).

The disproportion between the cow's genetically determined ability to produce milk and the limitations on improving the energy value of the ration may be the cause of the metabolic stress of animals kept at organic farms. Negative energy balance has a major impact on the body's hormonal balance and the functioning of the organs and it also affects the content of basic components (protein, fat and lactose) of milk cows. Physiological changes in the cow's body, which occur during lactation, might have significant influence on the biological value of milk and milk products. Little is known about the influence of lactation stage on the chemical composition and biological value of organic milk obtained from high-yielding Holstein-Friesian cows during pasture season.

The aim of the study was to investigate the influence of lactation stage on the biological value of organic milk obtained from high producing dairy cows during pasture season.

MATERIAL AND METHODS

Animals

The study was carried out on 68 Polish Holstein-Friesian primiparous at the Polish Organic Dairy Farm located in Juchowo (Western-Pomeranian Region). Juchowo Farm produces milk according to the legislation on organic farming issued by the Polish Ministry of Agriculture and Rural Development. All cows were handled in accordance with the regulations of the Polish Council on Animal Care, and the Polish Academy of Science Care Committee reviewed and approved the experiment and all procedures carried out in the study. Observations were conducted during two consecutive calendar years.

Treatments and pasture

For better representation, only cows calved in the spring season (lactating on pasture) were chosen for the experiment. The nutritional requirements of the animals and the nutritive value of their diet were calculated in accordance with the nutritional standards set out in the INRATION 4.0 software. The nutrient composition of the feedstuffs and diet are presented in Tables 1 and 2.

The feed intake for the organic production systems, including the pasture intake, was calculated according to standards based on the difference between herd demand and recorded intake of supplements. The use of additional vitamins as supplement to the feed was recorded.

During the study, cows were stripgrazed on a multispecies pasture (*Dac*tylis glomerata L., 12.8%; *Phalaris* arundinacea L., 4.2%; *Agrostis stoloni*fera L., 5.8%; *Poa pratensis* L., 17.5%;

TABLE 1. Nutrient composition of the diets at different stages of lactation	TABLE 1.	. Nutrient c	composition	of the	diets at	different	stages of lactation
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	Stage of lactation					
Component	BL (5–30 days post partum)	PL (60–90 days post partum)	FL (120–150 days post partum)	EL (250 days post partum)		
DM (%)	56.52	60.29	60.55	58.67		
Crude protein (CP) (% of DM)	14.55	14.42	14.54	14.71		
Acid detergent fiber (ADF) (% of DM)	24.40	29.11	29.76	28.50		
Neutral detergent fiber (NDF) (% of DM)	38.84	39.35	41.42	41.83		
Ether extract (% of DM)	3.86	3.98	4.30	3.89		
Ash (% of DM)	5.76	6.38	6.02	5.86		
Ca (% of DM)	0.53	0.48	0.51	0.47		
P (% of DM)	0.35	0.36	0.36	0.37		
UFL (kg of DM ⁻¹)	0.90	0.92	0.89	0.97		

	Stage of lactation					
Ingredient	BL	PL	FL	EL		
Ingredient	(5-30 days	(60–90 days	(120–150 days	(250 days post		
	post partum)	post partum)	post partum)	partum)		
Pasture with legumines (% of DM)	71.42	58.84	63.67	79.30		
Hay (% of DM)	_	12.07	18.86	7.42		
Total roughage (% of DM)	71.42	70.91	82.53	86.72		
Concentrates ¹ (% of DM)	27.64	28.05	16.50	12.27		
Supplements ² (% of DM)	0.92	0.96	0.94	0.99		
Total (offered) (kg of DM)	21.70	20.70	21.20	20.20		
Daily intake (kg)	17.80	19.20	19.80	20.10		

TABLE 2. Diet of experimental cow at various stage of lactation

Lolium perenne L., 19.4%; Festuca rubra L., 7.3%; Trifolium repens, 9.4%; Poa annua, P. trivialis, 8.3%; and broadleaf weeds, 10.3%; others 5.0%) at 7 AM to 4 PM every day. The cows received other feed or mineral supplements before evening milking and had ad libitum access to fresh water.

Measuring and sampling scheme

Animal performance parameters. 272 milk samples were collected from cows which were in one of the following phases (LS): the beginning of lactation (BL): 5–30 days post partum; peak of lactation (PL): 60–90 days post partum; full lactation (FL): 120–150 days post partum; end or final phase of lactation (EL): 250 days post partum. The cows were milked daily at 5.30 AM and 5.30 PM and milk yield was recorded at each milking. During the experiment milk samples were obtained twice from each cow from the morning and evening milking (according to sampling scheme). Milk was

placed in sterile bottles, preserved with Mlekostat CC and immediately submitted to the Cattle Breeding Division (Milk Testing Laboratory of WULS) for composition analysis. The basic parameters of the milk, i.e. fat, protein, lactose, urea content, were determined by automated infrared analysis with a Milkoscan FT – 120 instrument (Foss Electric; Hillerod, Denmark). An evaluation of the hygienic status of the milk was based on somatic cell count using the Somacount – 150 (Bentley, Warsaw, Poland).

Whey proteins. The determination of lactoferrin concentration were established using an Agilent 1100 Series reverse phase high-performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) according to the methodology described by Puppel et al. (2014).

Vitamins soluble in fat and β -carotene. Analysis of the fat soluble vitamins: β -carotene and α -tocopherol were established using an Agilent 1100

¹Concentrates: sum of cereals, by-products, and commercial concentrate mix.

 $^{^2}$ Supplements: calcium 18%, phosphorus 7%, sodium 8.2%, magnesium 4.5%, vitamin A 1,000,000 IU, vit. D₃ 100,000 IU, vitamin E 3000 mg, manganese 3000 mg, zinc 9000 mg, copper 1200 mg, iodine 100 mg, cobalt 20 mg, selenium 40 mg.

Series reverse phase high-performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) and Zorbax Eclipse XDB C8 column (4.6×150 mm, $5 \mu m$ film thickness) according to the method described by Puppel et al. (2013).

Blood. Blood samples were collected from the animal's tail vein by an authorized veterinarian (empty test tubes, for clotted blood, and test tubes containing lithium heparin were used). Blood was collected before the morning feeding. After collection the samples were transported on ice, at temperatures around 0–6°C. In the laboratory blood was centrifuged at 3500 RPM for 10 min and put to Eppendorf type tubes of 1.5 ml capacity, and then frozen at –30° C for further analysis.

Levels of the following blood parameters were estimated with the help of UV-VIS spectrophotometer ULTRO-SPEC 2000 (PHARMACIA BIOTECH) in the blood serum probes: β-hydroxybutyric acid (BHBA), enzymatic method, the BHB RANBUT test (Randox), aspartate aminotransferase (AST), colorimetric method, AST test (Pointe Scientific) and alanine aminotransferase (ALT), colorimetric method, ALT test (Pointe Scientific), non-esterified fatty acids (NEFA), colorimetric method, NEFA test (Pointe Scientific).

Statistical analysis

The data obtained were analyzed statistically using a ANOVA procedure by means of the SPSS 12.0 packet software (SPSS, Chicago, IL, USA). The mathematical model that describes the influence of lactation stage on analyzed traits is given by

$$Y_{ij} = \mu + \tau_i + \ddot{o}_{ij}$$

where:

 Y_{ii} – the *j*-th observation ($j = 1, 2, ..., n_i$);

μ – common effect for the whole experiment;

 τ_i – the *i*-th lactation stage effect ((*i* = BL (5–30 days), PL (60–90 days), FL (120–150 days), EL (> 250 days));

 \ddot{o}_{ij} – the random error present in the *j*-th observation on the *i*-th stage of lactation.

The level of significance was determined after performing preliminary statistical analyses.

RESULTS AND DISCUSSION

Milk production is a function of the number and activity of mammary epithelial cells, regardless of lactation stage. The highest level of protein was observed in EL. A similar trend was noted in the case of fat concentration. This was probably due largely to the concentrating effect of decreasing milk volumes, since yields of fat and protein decreased with advancing lactation (Table 3). DePeters and Cant (1992) observed that the reduction in protein concentration in milk is often caused by the dilution effect, rather than its reduced synthesis in the mammary gland, and is always associated with an increase in milk yield. Similar results were reported by Auldist et al. (1998) and Dewhurst et al. (2006). The lowest fat, protein and dry matter content in PL was probably caused through the use of energy from adipose tissue in the BL (Pedron et al. 1993). At this time the cow cannot satisfy its energy needs from feed intake alone, which is still too small

	Stage of lactation					
	BL	PL	FL	EL		
Item	(5–30 days <i>post</i>	(60–90 days	(120-150 days	(250 days		
	partum)	post partum)	post partum)	post partum)		
	LSM1 (SEM2)	LSM (SEM)	LSM (SEM)	LSM (SEM)		
Sample number	68	68	68	68		
Milk yield (kg·d ⁻¹)	22.7 ^{Ac} (3.05)	23.5 ^{Bd} (3.58)	18.3 ^{cd} (3.21)	15.8 ^{AB} (3.59)		
Fat (g·kg ⁻¹)	42.8a(1.1)	38.5 ^b (1.30)	43.4a(1.40)	43.5 (1.60)		
Protein (g·kg ⁻¹)	30.9 ^{Aa} (0.40)	29.3 ^{Ab} (0.50)	30.5 ^A (0.50)	33.1 ^B (0.60)		
Lactose (g·kg ⁻¹)	46.9 (0.20)	47.6 (0.30)	47.1 (0.30)	47.5 (0.40)		
Somatic cell count (10 ³ ·ml ⁻¹)	95.72a (12.59)	53.09a (11.47)	62.66 (13.25)	69.82 (12.89)		
Urea (mg·l ⁻¹)	270.5a (18.45)	285.4 (20.32)	279.1 (21.45)	259.5ª (23.65)		

TABLE 3. Changes in milk performance of analyzed cows during different stages of lactation

(Reist et al. 2002) in this lactation phase. Milk lactose percentages were, however, very similar for all periods. The content of lactose in milk is subject to the smallest changes. The higher content of lactose in organic milk can be explained by the higher concentrations of sugar in feed grasses on organic farms. In BL cows primarily mobilize body fat. Nevertheless, a limited amount of body proteins will be mobilized as well and can result in elevated plasma urea concentrations.

The content of urea is influenced by the following factors: feed composition and dose, the weight of cows, milk yield, season, month and lactation period. During pasture feeding high levels of urea can be tolerated and even economically justified. Cows grazed *ad libitum* on young pasture (excess of easily degradable protein) consume much more protein than they can turn into milk. Therefore, the pasture grazing cows may produce milk with a high level of urea (even higher than 300 mg·l⁻¹). The urea content

in organic milk samples ranged between 259.5 and 270.5 mg·l⁻¹ (Table 3). Urea and protein content in milk are indicators of a diet balanced in terms of energy and protein, so if the protein content is within normal ranges (3.2–3.8%), and urea concentration is between 15–30 mg and 100 m·l⁻¹, it is considered that the energy and crude protein supply are at an optimum level (Bendelja et al. 2011).

The main source of fat-soluble vitamin in milk fat is feed. The concentration of β-carotene in milk depends on the level of β-carotene in feed and on the breed of cow. Reported by Prache et al. (2003), β-carotene content in pasture herbage collected between May and June fluctuated between 620–720 mg·kg⁻¹ of DM, and was significantly reduced in late August to 430 mg kg⁻¹ DM. In the BL the lowest content of fat-soluble vitamins in milk were observed. At the same time, the BHBA concentration increased, which may confirm energy demand for fast growing milk yield in the first lacta-

¹ SEM – standard error of the mean; ² LSM – least square of mean; values in the same row marked with the same letters differ significantly $^{AA, BB}$ at $P \le 0.01$; $^{aa, bb}$ at $P \le 0.05$.

	Stage of lactation					
	BL	PL	FL	EL		
Item	(5–30 days	(60–90 days	(120–150 days	(250 days		
	post partum)	post partum)	post partum)	post partum)		
	LSM ¹ (SE ²)	LSM (SE)	LSM (SE)	LSM (SE)		
Lactoferin (g·l-1)	0.330 (0.001)	0.283 (0.001)	0.147 (0.001)	0.158 (0.001)		
Vitamin E (μg·dm ⁻³)	1339.7 (15.668)	1954.1 (19.896)	28774.7 (25.357)	2411.4 (22.158)		
β-carotene (μg·dm ⁻³)	349.95 (5.365)	598.5 (7.658)	701.6 (6.568)	483.3 (6.547)		
BHBA (mmol·dm ⁻³)	0.613 ^A (0.045)	0.420 ^A (0.025)	0.440 ^A (0.034)	0.421 ^A (0.029)		
AST (U·dm ⁻³)	51.75 ^A (5.895)	49.1 ^B (6.346)	57.25 ^{AB} (4.985)	56.75 ^{AB} (5.678)		
ALT (U·dm ⁻³)	14.05 ^A (0.345)	20.2 ^A (0.457)	22.15 ^A (0.587)	22.25 ^A (0.398)		
NEFA (g·0.1 kg of milk fat-1)	0.475 ^A (0.004)	0.580 ^b (0.003)	0.845 ^{Ab} (0.007)	0.912 ^{Ab} (0.008)		

TABLE 4. Changes in analyzed components of milk and blood during different stages of lactation

tion phase (Table 4). The highest level of β-carotene in milk was achieved in the EL (701.6 μg·dm⁻³). The highest vitamins E and A level was observed in the FL, where energy could be used for synthesis of the antioxidants. Dairy cattle consuming stored forages are often low in vitamin E, and vitamin E deficiencies are frequently observed during the periparturient period. Vitamin E can quench peroxidation reactions in cell membranes and is probably the most important antioxidant located in membranes (Putnam and Comben 1987). In cows near calving, Paterson (1965) reported a positive relationship between NEFA and the carotenoid content of plasma, suggesting the ability of bovine adipose tissue to release β-carotene.

β-hydroxybutyrate acid (BHBA), along with acetone and acetoacetate, is considered a ketone. Ketones are produced during the metabolism of non esterified fatty acids (NEFAs) and volatile fatty acids. BHBA values above 27 mg·dl-1 are considered compatible with clinical ketosis. Cows with underlying hepatic lipidosis may have concurrent elevations in liver leakage enzymes: AST or cholestatic enzymes ALP. The level of BHBA concentration in blood is an indicator of a positive or negative energy balance in the cow. Additionally, cows with BHB values above or equal to 12.5 mg·dl⁻¹ (1200 μmol·l⁻¹) were eight times more likely to experience a left-displaced abomasum. The highest level of BHBA was observed in BL – 0.615 mmol·dm⁻³ (Table 4). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important catabolic enzymes, which play an important role in the liver function of animals. Alanine aminotransferase activity in cows differs during certain production periods. The lowest ALT activity was measured during BL, while activity increased in the PL, FL, EL (Table 4). The aspartate aminotransferase activity changes irregularly during lactation. NEFA concentration reflects

¹ SEM – standard error of the mean; ² LSM – least square of mean; values in the same row marked with the same letters differ significantly AA, BB at $P \le 0.01$; aa, bb at $P \le 0.05$.

the mobilization of lipid reserves to compensate for the imbalance between nutrients consumed by the cow and nutrients secreted in milk. Serum NEFA greater than 0.4 mEq·l-1 has been proposed to identify excessive prepartum NEB. NEFA concentrations were relatively low because energy balance becomes positive and cows replete the mobilized tissue reserves. The highest concentration was found in the EL (22.5 U·dm⁻³) and the lowest in the BL (Table 4). NEFA respond much guicker than BHBA to homeostasis changes. The emergence of an energy deficit results in a rapid increase of blood NEFA, and its rapid decline. Blood levels of NEFA usually show a strong variation related primarily to time of feeding. Radloff et al. (1966) noted that plasma NEFA levels decreased at 4 to 6 h after feeding while blood ketones increased.

CONCLUSION

Stage of lactation had a significant effect on the most analyzed milk constituents including: whey proteins, fat soluble vitamins, phospholipids. The study has shown a significant influence of lactation stage on the biological value of organic milk obtained from high producing dairy cows during pasture season. In addition, there is evidence that the organic intensity of the production system has no influence on the metabolic profile of high yielded cow's as the plasma parameters were in norm.

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Streszczenie: Wpływ stadium laktacji wysokowydajnych krów rasy PHF na kształtowanie się parametrów profilu metabolicznego krwi oraz bioaktywnych składników ich mleka w trakcie sezonu pastwiskowego w ekologicznym systemie produkcji. Dysproporcja między zapotrzebowaniem bytowym krów a możliwościa jego pokrycia powoduje obciążenie metaboliczne organizmu. W przypadku krów mlecznych główna przyczyna obciążenia metabolicznego jest brak równowagi między genetycznie uwarunkowanym potencjałem dużej produkcji mlecznej a ograniczonymi możliwościami pokrycia potrzeb pokarmowych energią zawartą w pobranych paszach. Skutkuje to ujemnym bilansem energii i nadmierna mobilizacją tłuszczu z rezerw organizmu, jak również zmianą w koncentracji bioaktywnych składników frakcji białkowej oraz frakcji tłuszczowej. Celem pracy było określenie wpływu stadium laktacji na kształtowanie się podstawowego składu chemicznego mleka oraz profilu metabolicznego wysokowydajnych krów w ekstensywnym systemie żywienia. Badaniami objęto 68 krów rasy polskiej holsztvńsko-fryzyjskiej (pierwiastki). W trakcie realizacji doświadczenia pobrano 272 próbki mleka od krów, które znajdowały się w jednym z następujących stadiów laktacji: 5.-30. dzień laktacji (BL); 60.-90. dzień laktacji (szczyt laktacji; PL); 120.-150. dzień laktacji; powyżej 250. dnia laktacji (EL). Badania wykazały, że faza laktacji w istotny sposób kształtowała koncentrację laktoferyny, witaminy E oraz β-karotenu w mleku krów w trakcie sezonu pastwiskowego. Najwyższa koncentrację laktoferyny wykazano w początkowym okresie laktacji (BL), podobną zależność wykazano w przypadku kwasu β-hydroksymasłowego (BHBA). Największą aktywność aminotransferazy alaninowej i wolnych kwasów tłuszczowych wykazano w końcowej fazie laktacji (EL). Wyniki

badań potwierdzają, że również w przypadku systemu ekstensywnego wzrost wydajności jest ściśle związany ze wzrostem zapotrzebowania krów na energię. Uzyskane wartości dla wskaźników profilu metabolicznego uzyskane dla czterech faz laktacji znajdowały się na niższym poziomie względem wartości uzyskiwanych przez krowy w intensywnym systemie produkcji. Dowodzi to, że stosowanie systemu ekologicznego gwarantuje zarówno lepszy pod względem wartości biologicznej produkt finalny, jak i zdrowe zwierzę (bez zaburzeń metabolicznych).

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