Relationship between milk yield, stage of lactation, and some blood serum metabolic parameters of dairy cows

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ABSTRACT: The aim of the study was to evaluate the effect of milk yield and stage of lactation on the activity of liver enzymes, cholesterol, and vitamin C concentration in blood of milking cows. The experiment was carried out on Polish Holstein-Friesian Black and White dairy cows with two different milk yield levels: M – medium (about 7000 kg per lactation) and H – high (about 10 000 kg per lactation). In blood serum, AST, ALT, GGT, CHOL, and vitamin C were estimated. The AST and ALT activities in the blood serum were lower in M group than in H group, however within M and H groups there were no differences in both aminotransferases activity between the 60th and the 200th day of lactation. Differences in GGT activity ($P \leq 0.01$), CHOL ($P \leq 0.05$), and vitamin C level ($P \leq 0.01$) in blood serum were found between both stages of lactation. Negative correlations between vitamin C level with somatic cell count and milk yield traits were observed, that may indicate an increase in oxidative processes in high-yielding dairy cows. The achieved results may be used in diagnostics and/or evaluation of herds from the point of view of biochemical and pathophysiological processes.

Keywords: cow milk; aminotransferase; cholesterol; vitamin C

The increase of milk production observed in dairy cows after calving is usually related to insufficient feed intake, which leads to negative energy balance (Drackley, 1999a, b). The energy demand of high-yielding cows in the peak of lactation exceeds the amounts of net energy from diet. This is a result of a decreased appetite after parturition and an increase in metabolic processes, which in consequences requires large amounts of substrates for milk constituents synthesis. A lack of sufficient nutritional components in the feed results in the use of body reserves, because in order to decrease the energetic deficiency, surplus fat is mobilized and undergoes lipolytic processes. Fat hydrolysis causes increased fatty acids metabolism in the liver, resulting in adipose tissue degeneration (Mulligan et al., 2006). Intensified lipolysis leads to metabolic disorders like hyperketonemia, hypoglycemia, and hyperbilirubinemia (Ingvarsten et al., 2003; Mulligan et al., 2006). These metabolic disorders negatively affect the yield, chemical composition, and technological parameters of milk (Rzewuska et al., 2011) and fertility traits as well (Roxtrom et al., 2000). Under conditions of energy deficiency also rumen functional disorders can be noticed. The function of the digestive tract organs determines the metabolic balance of the whole organism of a high yielding cow (Ingvarsten et al., 2003; Beever,

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2006). The homeostasis disturbance during this period is crucial for the performance of the dairy animals in the current as well as in the next lactations (Bertoni et al., 2009; Jóźwik et al., 2010b). The intensive energy turnover, under conditions of limited body reserves, causes high yielding cows to be more susceptible to numerous pathogenic factors, especially in early lactation (Ingvarsten et al., 2003; Litwińczuk et al., 2011). Biochemical blood indicators, like some liver enzymes activity in blood serum (γ-glutamyltransferase – GGT, aspartate aminotransferase – AST, alanine aminotransferase – ALT), are used for the evaluation of metabolic balance.

Some earlier studies showed relationships between milk yield with level of cholesterol and vitamin C in milk and blood (Ling et al., 2003; Chládek et al., 2004; Sordillo et al., 2009; Strzałkowska et al., 2009a, b). However, information about associations between biochemical parameters in blood and milking traits of dairy cows with different level of productivity is still not complete. Thus, the aim of the study was to evaluate the effect of milk yield and stage of lactation on the activity of liver enzymes and concentration of cholesterol and vitamin C in blood of milking cows.

MATERIAL AND METHODS

Animals

All procedures were performed according to the guiding principles for the care and use of research animals and were approved by the Local Ethics Commission No. 48/2005.

Table 1. Components of the diets based of feed rations (% of DM)

	TMR-1	TMR-2
Components		
Maize silage (35% DM)	32.64	27.97
Wilted grass silage (35% DM)	17.68	25.53
Barley straw	5.45	5.80
Corn maize silage	8.99	9.57
Soybean meal	8.86	–
Rapeseed meal	10.64	10.21
Triticale	8.86	19.09
Ketomix E-18[1]	4.19	–
Chalk	0.64	0.46
Co-bind A–Z[2]	0.20	0.21
Sodium bicarbonate	0.84	0.47
Witamix KW^{TM} [3]	1.01	0.69
Total	100.00	100.00
Feeding value of diets		
UFL/kg DM	0.97	0.93
PDI (g/kg DM)	105	86
Milk yield from energy in daily diet (kg/day)	40	34
Milk yield from protein in daily diet (kg/day)	48	37

UFL = feed unit for lactation, PDI = protein digested in the small intestine

[1]energy supplement (3.36 UFL/kg DM); [2]neutralizer of mycotoxins; [3]macro-elements (g/kg): Ca 120, P 60, Mg 50, Na 110; micro-elements (mg/kg): Mn 4000, Zn 9500, Cu 1150, J 90, Co 25, Se 45; vitamins (mg/kg): D_3 120 000, E 4000, K_3 50, B_1 150, B_2 100, B_6 50, B_{12} 550, calcium pantothenate 300, niacin 2500, folic acid 30, A 1000

Table 2. Amount of nutrients per cow in both diet groups

Diets	UFL (kcal)	Crude protein (g)	PDIN (g)	PDIE (g)	LFU (unit/ lactation)	ADF (g)	NDF (g)	P (g)	Ca (g)
TMR-1	22.33	3825	2553	2429	15.35	3843	1586	80.33	157.27
TMR-2	20.13	2892	1859	1875	15.64	3905	1533	62.27	109.13

UFL = 7200 kcal NEL, NEL = netto energy for lactation, UFL = feed unit for lactation, PDIN = protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-degraded organic matter, PDIE = protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-fermented organic matter, LFU = fill unit for lactating dairy cows, ADF = acid-detergent fibre, NDF = neutral-detergent fibre

The experiment was carried out on primiparous Polish Holstein-Friesian Black and White dairy cows (N = 88) with two different milk yield levels (M group = medium yielding cows, H group = high yielding cows). The animals were divided into four groups according to milk yield per lactation (M = 7000 kg and H = 10 000 kg) and lactation day (60 and 200) in the groups as follows:

1st group (M-DIM60): milk yield ca. 7000 kg per lactation, 60th day of lactation (N = 22)

2nd group (H-DIM60): milk yield ca. 10 000 kg per lactation, 60th day of lactation (N = 24)

3rd group (M-DIM200): milk yield ca. 7000 kg per lactation, 200th day of lactation (N = 20)

4th group (H-DIM200): milk yield ca. 10 000 kg per lactation, 200th day of lactation (N =22).

All animals were maintained at loose barn of the Institute Experimental Farm in Jastrzębiec, with free access to water and fed according to the INRA (National Institute for Agricultural Research, France) standards (Jarrige, 1989). Total Mixed Ration-1 (TMR-1) was fed to cows from groups M-DIM60, H-DIM60, and H-DIM200, whereas TMR-2 was fed to cows from group M-DIM200 (Table 1). The composition of the diets is presented in Table 2.

Analytical procedures

Milk samples were collected from the morning milking. Blood samples from jugular vein were collected by an authorized veterinarian before the morning feeding.

Aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), γ-glutamyltransferase (GGT; EC 2.3.2.2), and total cholesterol (CHOL) were estimated using COBAS INTEGRA® 400 plus system (Roche Diagnostics Ltd., Rotkreuz, Switzerland). The level of vitamin C in serum was determined according to Omaye (1979) using spectrophotometer LambdaBio-20 (Perkin Elmer, Waltham, USA). The chemical composition of milk was determined using IR-spectrophotometer Milkoscan™ FT2 (Foss, Hillerød, Denmark). The somatic cell count (SCC) was determined according to fluorescence microscopy method (IBC_m, Bentley Instruments, Inc., Chaska, USA).

Statistical analysis

Data were analyzed using GLM procedure of SAS, Version 8e for MS Windows (SAS, SAS/STAT 1999–2001) using the model which included days of lactation and the level of milk yield interactions. The procedure CORR was used to estimate the Pearson's correlations between biochemical indicators in serum with milk parameters.

RESULTS AND DISCUSSION

As expected, there were differences in milk yield traits between H and M groups in relation to milk performance during 305 days of lactation. Milk, fat, and protein yields were higher in H than in M group by about 52, 27, and 43%, respectively. However, fat content was lower ($P \leq 0.01$) in H than in M group (Table 3). No differences in milk protein content between groups were detected.

Milk yield was similar in M-DIM60 and M-DIM200 groups. However, there were differences in milk yield between M and H groups in both points of lactation ($P \leq 0.01$). Moreover, milk yield in M-DIM60 group was lower ($P \leq 0.01$) than in H-DIM200 group (Table 4). The results of the

Table 3. Milk yield and milk chemical composition (LSM ± SE) per 305-day lactation

Item	Medium		High	
	LSM	SE	LSM	SE
Milk yield (kg)	6953^{A}	245.65	10 574^{B}	298.87
Fat (kg/lactation)	273.9^{A}	14.65	346.8^{B}	15.35
Fat content (%)	3.90^{A}	0.23	3.30^{B}	0.21
Protein (kg/lactation)	237.3^{A}	12.89	339.5^{B}	13.34
Protein content (%)	3.40	0.23	3.22	0.22

^{A,B}within rows means bearing different superscripts differ at $P \leq 0.01$

study are in agreement with the results showed by Hickson et al. (2006) indicating negative correlation between lactation duration and peak yield in the Friesians.

The content of fat, protein, and total solids was the highest ($P \leq 0.05$) in M-DIM200. The lactose content and somatic cell count (SCC) remained on similar levels in milk of cows from all groups,

Table 4. Milk yield and milk chemical composition (LSM ± SE) according to studied periods and level of milk yield

Item	Medium				High			
	60-day		200-day		60-day		200-day	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Milk yield (kg)	22.1^{A}	3.53	20.2^{A}	3.13	37.9^{B}	3.30	32.1^{C}	3.30
FCM (kg)	20.6^{A}	3.63	19.2^{A}	4.25	33.5^{B}	3.67	31.1^{B}	4.27
VCM (kg)	25.0^{A}	3.73	23.8^{A}	3.79	38.5^{B}	4.49	38.0^{B}	4.13
ECM (kg)	20.73^{A}	3.42	21.12^{A}	3.07	34.51^{B}	3.13	29.53^{C}	3.51
Fat (%)	3.58^{a}	0.56	4.20^{b}	0.87	3.41^{a}	0.46	3.40^{a}	0.54
Protein (%)	3.27^{a}	0.30	3.68^{b}	0.34	3.18^{a}	0.30	3.32^{a}	0.29
Lactose (%)	4.97	0.20	4.92	0.19	4.99	0.10	4.95	0.17
Fat (kg)	0.88^{A}	0.07	0.78^{A}	0.05	1.18^{B}	0.07	1.15^{B}	0.08
Protein (kg)	0.82^{a}	0.06	0.71^{a}	0.05	1.16^{b}	0.06	1.08^{c}	0.05
Lactose (kg)	1.10^{a}	0.02	1.10^{a}	0.02	1.73^{b}	0.03	1.71^{b}	0.03
Urea (unit/l)	237.9	6.12	241.1	7.59	250.4	7.59	263.9	8.46
Total solids (%)	12.66^{a,b}	0.25	13.69^{b}	0.29	11.82^{a}	0.26	12.08^{a,b}	0.27
SNF (%)	9.05	0.24	9.22	0.25	8.84	0.25	9.14	0.25
Total solids (kg)	3.05^{a}	0.17	2.81^{b}	0.18	4.29^{c}	0.21	4.08^{c}	0.17
SNF (kg)	2.21^{A}	0.20	1.98^{A}	0.18	3.13^{B}	0.20	3.01^{B}	0.20
EBM (MJ/kg)	2.01^{A}	0.15	1.83^{A}	0.15	2.83^{B}	0.16	2.81^{B}	0.15
ln SCC	11.27	0.76	12.21	0.83	12.30	0.81	12.54	0.82

VCM = value corrected milk, FCM = fat corrected milk, ECM = energy corrected milk, SNF = solids non-fat, EBM = energy milk, SCC = somatic cell count

$^{a–c}$within rows means bearing different superscripts differ at $P \leq 0.05$

$^{A–C}$within rows means bearing different superscripts differ at $P \leq 0.01$

Table 5. Concentration of biochemical parameters (LSM ± SE) in serum according to studied periods and level of milk yield

Item	Medium				High			
	60-day		200-day		60-day		200-day	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
AST (unit/l)	55.92^{A}	0.08	53.76^{A}	0.13	75.36^{B}	0.15	76.59^{B}	0.17
ALT (unit/l)	39.54^{a}	0.06	39.47^{a}	0.12	47.14^{b}	0.11	50.65^{b}	0.13
GGT (unit/l)	38.23^{A}	0.07	46.18^{B}	0.08	36.86^{A}	0.10	56.82^{B}	0.15
CHOL (mg/100 ml)	146.15^{a}	0.20	$179.65^{b,c}$	0.32	171.43^{b}	0.28	212.82^{c}	0.35
Vitamin C (mg/100 ml)	1.18^{B}	0.01	1.75^{C}	0.01	0.96^{A}	0.01	1.28^{B}	0.02

AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = γ-glutamyltransferase, CHOL = total cholesterol

$^{a–c}$within rows means bearing different superscripts differ at $P \leq 0.05$

$^{A–C}$within rows means bearing different superscripts differ at $P \leq 0.01$

however high lactose content and the fairly low SCC in milk indicated quite good mammary gland health status (Čítek et al., 2011; Litwińczuk et al., 2011). The SCC did not exceed 200 000/ml in M groups and 280 000/ml in H groups.

The protein content in milk from cows of groups M-DIM60, H-DIM60, and H-DIM200 could indicate some energy deficiency. However, the protein content in M-DIM200 was high, what suggests that the energy demands of the animals were fully covered, but the high urea levels in all groups indicated the diets were not exactly balanced. Both high level of urea and low percentage of protein confirm that urea was not fully used for the milk protein production, which points to energy deficiency conditions (Řehák et al., 2009). The urea level exceeded the amount of 140 mg/ml considered as an accepted allowable maximum by some authors (Nousiainen et al., 2005).

Activity of AST, ALT, and GGT (Table 5) was higher than the accepted reference intervals 58–100, 25–74, and 22–64 units/l, respectively (Mordak, 2008). The AST and ALT activities in the blood serum were lower in M than in H groups, however within these groups there were no differences in both aminotransferases activity between points of lactation. Because the values of presented aminotransferases activity were included within reference intervals, the liver functions of high-yielding cows were not disturbed. The aminotransferases are responsible for the protein balance in the organism which is especially important in the period of intensive metabolism during the peak of lactation (Whitaker, 1997).

The GGT activity was lower on the 60th than on the 200th day ($P \leq 0.01$) in M and H groups. GGT is an enzyme which transfers a γ-glutamyl group from peptides and other compounds to an acceptor. An elevated serum GGT appears to be a sensitive specific indicator of liver damage, making it a useful diagnostic aid.

The lowest cholesterol content in serum was found in M-DIM60 group and the highest in H-DIM200 ($P \leq 0.05$). In both M and H groups the cholesterol level was lower on day 60 than on day 200. Similar results were observed by Ling et al. (2003). They found that cholesterol content was the lowest in early stage of lactation and then increased gradually during lactation.

The results show also the effect of milk yield and SCC on serum ascorbic acid concentration. Vitamin C content was higher in the blood serum of M than H group and in both the groups the vitamin content increased with the lactation duration. Vitamin C is considered as the most important antioxidant found in mammals' organisms. Its highest concentration occurs in neutrophils, which play important role in the innate immunity to mastitis (Weiss et al., 2004; Jóźwik et al., 2010a).

In the present study negative correlations between vitamin C with SCC as well as yield traits were observed (Table 6). Cows with a mammary gland infection exhibited lower concentrations of vitamin C in plasma than did healthy cows (Weiss et al., 2004). In addition, Weiss et al. (2004) observed significant correlations between vitamin C concentrations in plasma and milk and clinical signs of mastitis caused by *E. coli*.

Table 6. Pearson's correlations between biochemical indicators in serum and milk parameters

Trait	Vitamin C	CHOL	GGT	AST	ALT
SCC	–0.494*	ns	ns	ns	ns
Protein (%)	ns	ns	ns	ns	ns
Lactose (%)	ns	ns	ns	ns	ns
Fat (%)	ns	ns	ns	ns	ns
Daily milk yield (kg)	–0.397*	0.403*	ns	0.353*	0.423*
Total solids (%)	ns	ns	ns	ns	ns
NFDM (%)	ns	ns	ns	ns	ns
Urea (unit/l)	ns	ns	ns	ns	ns
Protein (kg)	–0.419*	0.539**	ns	0.381*	0.577**
Lactose (kg)	–0.387*	0.397*	ns	0.351*	0.448*
Fat (kg)	ns	0.317*	ns	0.386*	0.571**
Dry matter (kg)	–0.378*	0.448*	ns	0.359*	0.418*
NFDM (kg)	–0.403*	0.456*	ns	0.367*	0.502**
FCM (kg)	–0.311*	0.376*	ns	0.399*	0.542**
VCM (kg)	–0.373*	0.502**	ns	0.400*	0.611**
ECM (kg)	–0.361*	0.439*	ns	0.387*	0.491*
EBM (MJ/kg)	–0.413*	0.538**	ns	0.376*	0.576**
Vitamin C (mg/100 ml)		ns	ns	ns	ns
CHOL (mg/100 ml)			0.446*	ns	0.460*
GGT(unit/l)				ns	ns
AST (unit/l)					0.721**

SCC = somatic cell count, NFDM = non-fat dry matter, VCM = value corrected milk, FCM = fat corrected milk, ECM = energy corrected milk, EBM = energy milk, CHOL = total cholesterol, GGT = γ-glutamyltransferase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ns = non-significant

*$P \leq 0.05$, **$P \leq 0.01$

The high-producing cows have a great demand for glucose to synthesize lactose and thus may insufficiently produce ascorbic acid (Macleod et al., 1999). Negative correlation between vitamin C with milk parameters might point to the increase of oxidative processes in high-yielding dairy cows (Sordillo et al., 2009).

Glucose is essential in the production of proteins and in lipid metabolism. In almost all mammals including cows, it is also a precursor for vitamin C (ascorbic acid) production. In cells glucose is converted into ascorbic acid with the help of the enzyme gulonolactone oxidase (Padayatty et al., 2003).

The AST and ALT activity in the blood serum of cows in the presented study fitted between the reference levels and was positively correlated with daily milk yield, FCM, VCM, ECM, EBM, fat, protein, and lactose (0.35–0.40 and 0.42–0.61 for AST and ALT, respectively, $P \leq 0.05$; $P \leq 0.01$) (Table 6).

Similar results regarding to aminotransferases activity were obtained by Dobranić et al. (2006). In our study also the positive correlations between yield traits with cholesterol content in blood serum were estimated. Mohebbi-Fani et al. (2009) also found the positive correlations between cholesterol with uncorrected or corrected milk yield during several periods of lactation.

Our results indicated that the milk yield affects AST and ALT activities as well as cholesterol level. The relationships between cholesterol concentra-

tion in blood plasma and milk production was analyzed by Chládek et al. (2004) in Holstein dairy cows but the correlation between cholesterol content in blood plasma with milk production parameters did not reveal a clear relationship. However, the graphical expression showed the tendency towards a positive relationship between blood plasma cholesterol and milk yield and a negative one between blood plasma cholesterol and milk protein content. However, no relationship between blood plasma cholesterol and milk fat content was found due to a high variability of fat content in milk.

During the early stage of lactation the liver of high productivity cows undergoes extensive physiological and biochemical changes to counteract the adverse effects of negative energy balance (Stojević et al., 2005). The correlations between protein, lactose, fat, and dry matter yields with AST and ALT activity obtained in our study confirmed this thesis.

Because the liver has a variety of biochemical, synthetic, and excretory functions, several biochemical tests, called liver-function tests, are used in the diagnostics and management of liver diseases. Determination of biochemical parameters in blood serum can provide valuable information regarding dairy cows nutrition and physiological status in relation to age and stage of lactation.

CONCLUSION

The obtained results show the influence of milk yield and stage of lactation on the activity of liver enzymes, cholesterol, and vitamin C concentration in blood of high yielding cows. The values of serum activity of liver enzymes, particularly AST and ALT, are in the accepted range of reference values. This indicates that hepatocyte cellular was not damaged. Under conditions of full supply of cows' nutritional requirements the high milk yield characterized by optimal chemical composition and SCC level can be achieved. The results of the present study may hopefully contribute to diagnostics and/or evaluation of herds from the viewpoint of biochemical and pathophysiological processes.

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