The relationship between milk fat to protein ratio and selected production traits of Polish Holstein-Friesian cows

Piotr Guliński*, Stanislaw Socha

Siedlee University of Natural Sciences and Humanities, Institute of Animal Production and Fisheries, Siedlee, 08-110 Siedlee, Prusa 14, Poland

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The aim of this paper was to assess the relationship between the fat-protein ratio and selected characteristics of milk from Polish Holstein-Friesian (PHF) cows. Altogether, 891 milk samples from the first two months of lactation (60 days) were included in the analysis. Nine milk production traits were recorded: raw milk yield (kg), FCM - fat corrected milk yield (kg), FPCM - fat and protein corrected milk (kg), percentage of fat, protein, dry matter, and lactose, urea level (mg/L), and somatic cell number (thousand/mL). Cows were divided into four groups according to the fat to protein ratio (F/P ratio): ≤ 1.1 : 1 indicating rumen acidosis; 1.1-1.4: 1 optimal; 1.4-1.7: 1 indicating subclinical ketosis; >1.7 : 1 indicating clinical ketosis. The results demonstrated that ketosis and consequently the fat protein ratio affected milk production traits. A decrease in the ratio from 1.1-1.4 : 1 to >1.7 : 1 meant the daily milk yield lower by 3 kg, the lactation milk yield of 305 days lower by 649 kg, and lower protein and lactose content by 0.16 and 0.09%, respectively. The high daily milk yield loss was related to an increase in the F/P ratio in milk. Each additional 0.2 increase in the F/P ratio (beyond 1.0 : 1) resulted in a 1.52 kg milk loss for the first 60 DIM (days in milk) and 352 kg for 305 DIM. Similarly, in the first 60 DIM each 0.2 increase in the F/P ratio led to an increase in fat content in milk by 0.63% and a decrease in protein and lactose content by 0.05 and 0.02%, respectively. Generally, the results of this study show that the occurrence of ketosis in dairy herds leads to significant changes in the chemical composition of milk. Another effect is the reduction of milk yield in lactation.

KEYWORDS: dairy cattle / fat-protein ratio / ketosis / milk composition

^{*}Corresponding authors: piotr.gulinski@uph.edu.pl

One of the consequences of milk production intensification is the aggravation of the effects of the negative energy balance during the initial lactation period. In situations of negative energy balance (NEB), ketosis occurs, manifested by an increase in the level of ketone bodies in the body fluids of animals and by a number of clinical symptoms. The most important of these include decreased appetite, preference for roughage, the smell of acetone in the exhaled breath and in urine, development of other diseases (mastitis, metritis, displaced abomasum), disturbance of reproduction and drop in milk production. Other characteristic symptoms of the disease are connected with an increase in the level of fat in milk (above 5%) and a decrease in protein levels to less than 2.9%.

The gold standard method for ketosis testing in dairy cows is based on measuring the levels of blood β -hydroxybutyric acid (β HBA) [Djoković *et al.* 2019, Duffield *et al.* 2009, Iwersen *et al.* 2009]. As an invasive and expensive method it is primarily used by veterinarians for the diagnosis of clinical ketosis. In most cases only cows with pronounced clinical signs are subjected to blood analysis [Guliński 2020].

In practice, due to its positive correlation with a degree of negative energy balance the fat-to-protein ratio (F/P) has been proposed as a method to diagnose subclinical ketosis (SCK) in dairy cows [Duffield *et al.* 1997, Hauer *et al.* 1999]. Duffield *et al.* [1997] observed that the best cutoff point to diagnose subclinical ketosis (β HBA \geq 1.2 mmol/L) in the first 65 days in milk (DIM) was F/P >1.33, but the sensitivity and specificity of the F/P ratio were only 58 % and 69%, respectively. Heuer *et al.* [1999] used a cutoff point of F/P >1.5 at the first monthly milk recording after calving and observed that cows with F/P >1.5 had an increased risk for clinical ketosis (CK), displaced abomasum, ovarian cysts, lameness and mastitis.

Jenkins *et al.* [2015] studied the F/P and cutoff points used to diagnose SCK. For the following F/P cutoff points: >1.25, 1.35, 1.50, 1.60, and 1.70, the sensitivity and specificity of the fat to protein ratio was 100 to 49, 96 to 59, 75 to 78, 33 to 90, and 8 to 96%, respectively. The proportions of cows herds with F/P >1.25, 1.35, 1.42, 1.50, 1.60, and 1.70 were 60, 50, 44, 30, 14, and 6%, respectively.

The effects of the dairy cow F/P ratio have been dealt with in several studies [Buttchereit *et al.* 2012, Toni *et al.* 2011, Zink *et al.* 2014]. Buttchereit *et al.* [2012] reported that the F/P ratio and body condition score are potential variables to determine how well cows can adapt to early lactation challenges. An increase in the F/P ratio during early lactation is also statistically significantly related to an increase in the risk of culling [Toni *et al.* 2011]. Moreover, the F/P ratio is a valuable indicator of negative energy balance in postpartum cows and might be helpful in selecting cows for culling due to their metabolic or other disorders [Zink *et al.* 2014]. Additionally, Buttchereit *et al.* [2010] reported that the F/P ratio reflects the energy balance status of a cow in early lactation.

Deleterious effects of ketosis on animal health, production, and reproduction have been studied by a number of researchers. The negative effects of SCK on animal production were described by several authors [Chapinal *et al.* 2012, Gantner *et al.* 2016, McArt *et al.* 2012, Oetzel 2007, 2012, Rajala-Schultz *et al.* 1999]. The effect of early lactation NEB, with the disruption of the hypothalamus-pituitary-ovary axis, on later reproductive performance is also documented [Butler, 2003]. Other studies confirm the negative effects of SCK on reproductive efficiency [McArt *et al.* 2012, Ospina *et al.* 2010b, Walsh *et al.* 2007]. Clinical ketosis results in 2 to 3 more days to first service and a 4 to 10% reduction in pregnancies per AI (artificial insemination) at the first service. According to Rutherford *et al.* [2016], the first insemination was 4.3 times less likely to be successful in SCK cows than in non-SCK cows. The adjusted mean number of inseminations per pregnancy was 2.8 for SCK cows and 2.0 for non-SCK cows. Other researchers have identified a link between ketosis and an increased incidence of ovarian cysts.

Steeneveld *et al.* [2020] calculated total costs of clinical and subclinical ketoses under current economic conditions in the Netherlands. Average herd level costs of ketosis (CK and SCK combined) were €3613 per year for a default farm and €7371 per year for a high-risk farm. The costs for a single CK case were on average €709, while the costs for a single SCK case were on average €150 for default farms. According to Mostert *et al.* [2018], total costs of SCK were €130 per case per year, with a range between €39 and €348. Total costs of SCK per case per year resulted from a prolonged calving interval (36% of all costs), reduced milk production (24%), treatment (19%), discarded milk (14%), and removal (6%).

The aim of the study was to assess the relationships between the fat to protein ratio in milk and selected traits of Polish Holstein-Friesian cows.

Material and methods

Selected traits related to the chemical and cytological quality of milk produced by 10 dairy herds located in eastern Poland were studied. With 891 daily observations, the studies involved the evaluation of milk performance parameters of 586 Polish Holstein-Friesian (PHF) cows during their first two months (60 days) of lactation. These animals were of different ages (completed different lactations) and had different F/P ratio values in milk. The herds included in the study belonged to typical dairy herds in eastern Poland. The average number of cows per herd was 49. The cows were kept in tie-stall (8) and free-stall (2) barns. In the feeding of cows in individual herds, the TMR (Total Mixed Ration) and PMR (Partly Mixed Ration) technologies were used. The assessment of the F/P ratio and the daily milk yield, the content of selected milk components and the correlation between these features concerned the first 60 days of lactation. The exception was milk yield for a 305-day lactation, the level of which was correlated with the F/P ratio in milk obtained during the first two months of lactation. The following milk quality traits were recorded: raw milk yield (kg); fat corrected milk yield FCM (kg); fat protein corrected milk FPCM (kg); the percentage of fat, protein, dry matter, and lactose; urea level (mg/L) and the somatic cell count (thousand/mL). The somatic cell count (SCC) was log-transformed to the somatic cell

score (SCS) as follows: SCS = log2(SCC/100,000)+3 [Wiggans and Shook, 1987]. Individual daily raw milk yields during the first two months of lactation and during 305 days of lactation were corrected for 4% fat content (FCM) using the formula of 4% FCM = $0.4 \times \text{milk yield (kg)} + 15 \times \text{fat yield (kg)}$ [Gains, 1928] and for 4% fat and 3.3% protein content (FPCM) using the formula FPCM= milk yield (kg) × (0.337 + 0.116 × fat (%) +0.06 × protein (%)) [Yan *et al.* 2011].

In the first stage the following four milk fat to protein F/P ratio groups were created: $\leq 1.0:1$ indicating rumen acidosis; 1.1-1.4 : 1 optimal; 1.4-1.7 : 1 indicating subclinical ketosis; > 1.7 : 1 indicating clinical ketosis.

Several factors affecting the relationship between the F/P ratio and milk production traits were studied. There were four age groups (1 - first lactation, 2 - second lactation, 3 - third and fourth lactations, 4 - fifth to ninth lactations); 10 cow herds (1-10); three classes of daily milk yield - <20 kg, 20-30 kg, >30 kg. Detailed data on the number of animals and records within each class are presented in Table 1 and 2.

A linear model containing effects of the age of cows, herd number, daily milk yield class, and F/P ratio groups was used:

$$y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + F_m + (AB)_{ij} + (AC)_{ik} + (AD)_{il} + e_{ijklmn}$$
where:

 y_{iiklmn} - the *ijklmn-th* observation of the trait;

 μ – overall mean;

 A_i - fixed effect of the *i*-th F/P ratio group (i = 1, 2, 3, 4);

 B_{i} - fixed effect of the *j*-th herd (j=1-10);

 C_k - fixed effect of the *k*-th cow age (k=1,2,3,4);

 D_{l} - fixed effect of the *l*-th daily milk production level (l=1,2,3);

 F_{m} - random effect of the m-th cow (i=1-586);

 $(AB)_{ii}$ - effect of the F/P ratio by herd interaction;

 $(AC)_{ik}$ - effect of the F/P ratio by cow age interaction;

 $(AD)_{il}$ – effect of the F/P ratio by daily milk production level interaction;

 e_{iiklmn} - random error connected with *ijklmn*-th observation.

The relationships between the F/P ratio and daily milk yield were determined by the Pearson correlation and linear regression. Regression equations were calculated to relate the F/P ratio and selected milk production traits, e.g. daily milk yield (kg), 305-day milk yield (kg), fat (%), protein (%), dry matter (%), lactose (%), urea (mg/l) and SCS. Linear regression coefficients of dependent variables (daily milk yield, 305-day milk yield, fat, protein, dry matter, lactose, urea and SCS) on the F/P ratio were calculated.

The results were statistically processed applying the multi-way analysis of variance with the least squares method. Significance of differences between means was estimated with the Duncan test at P \leq 0.01. The computations were performed using the GLM and REG procedures of the SAS statistical package [SAS Institute, 2008].

Results and discussion

Table 1 contains the results for the effects of the F/P ratio on milk yield and composition. Significant effects of ketosis and a related increase in the F/P ratio on milk characters were estimated In the studied population, an increase in the F/P ratio from 1.1-1.4 : 1 to >1.7 : 1 led to a decrease in daily milk yield, protein and lactose percentage, and 305-day lactation milk yield by 3 kg (p<0.001), 0.16% (p<0.001), 0.09% (p<0.001), and 649 kg (p<0.001), respectively. On the other hand, an increase in the fat-protein ratio from 1.1 to 1.4 : 1 to >1.7 : 1 resulted in an increase in the percentage of fat. In addition, a decrease in urea levels in milk with the increased F/P ratio was observed. In the group of cows with the normal fat-to-protein ratio (1.1-1.4

		F/P ratio				
Item		daily measurements (first 60 days lactation)				P-value
		<u>≤</u> 1.1 : 1	1.1-1.4 : 1	1.4-1.7 : 1	≥1.7 : 1	
No observation		148	382	271	89	
No of cows		116	245	153	72	
Milk yield (kg)	mean (SD)	32.7 ^a (11.1)	$28.6^{\text{B}}(8.4)$	25.8 ^c (7.1)	$25.6^{\circ}(6.8)$	< 0.001
with yield (kg)	min. – max.	9-58.8	12-60.0	13.8-49.3	11.2-46.2	<0.001
FCM (kg)	mean (SD)	28.2 ^B (9.2)	28.1 ^B (8.1)	28.2 ^B (7.7)	31.9 ^A (9.3)	0.001
FCM (Kg)	min. – max.	6.6-51.4	11.5-56.9	14.2-64.7	14.3-63.9	0.001
FPCM (kg)	mean (SD)	28.9 ^{A,B} (9.5)	27.9 ^B (8.1)	27.3 ^B (7.4)	29.9 ^A (8.5)	0.029
rrcwi (kg)	min max.	7.1-53.1	11.4-57.1	13.7-62.9	13.6-57.0	0.029
Fat(0/)	mean (SD)	$3.11^{D}(0.54)$	$3.90^{\circ}(0.46)$	$4.62^{B}(0.54)$	$5.63^{A}(1.02)$	< 0.001
Fat (%)	min max.	1.73-4.92	2.79-5.42	3.5-6.76	3.87-9.87	<0.001
Protein (%)	mean (SD)	3.21 ^A (0.47)	$3.12^{\text{B}}(0.31)$	$3.06^{\text{B}}(0.32)$	2.96 (0.33)	< 0.001
FIOLEIII (70)	min max.	2.44-6.01	2.43-4.3	2.41-4.4	2.25-4.13	<0.001
Dere metter (0/)	mean (SD)	11.83 ^D (0.92)	$12.54^{\circ}(0.95)$	13.23 ^B (0.81)	$14.02^{A}(1.14)$	< 0.001
Dry matter (%)	min max.	8.75-15.89	5.05-17.94	11.17-15.71	12.35-18.0	<0.001
Lactose (%)	mean (SD	$4.80^{A}(0.29)$	4.82 ^A (0.21)	$4.82^{A}(0.20)$	$4.73^{B}(0.36)$	< 0.001
Lactose (76)	min max.	2.64-5.23	2.94-5.36	4.14-5.22	3.49-5.96	<0.001
Unas (mag/I)	mean (SD)	207 ^A (74)	198 ^{AB} (76)	$182^{B}(79)$	$186^{\rm B}(76)$	0.005
Urea (mg/L)	min max.	71-475	1-426	1-453	1-475	0.005
SCC	mean (SD)	471 (1060)	357 (739)	350 (856)	610 (1670)	0.089
scc	min. – max.	7-8302	5-6373	6-9137	15-1000	0.089
SCS	mean (SD)	4.89 ^{AB} (1.51)	4.84 ^B (1.39)	$4.76^{\text{B}}(1.36)$	5.17 ^A (1.38)	0.107
303	min max.	1.94-9.02	1.60-8.75	1.79-9.12	2.70-9.21	0.107
		lactation measurements				
305 day milk	mean (SD)	7915 ^A (2518)	6986 ^B (2182)	6177 ^c (1707)	6337 ^c (1852)	< 0.001
yield (kg)	min max.	2382-13626	2382-14431	2382-12775	2746-11673	<0.001
	mean (SD)	7553 ^A (2275)	7030 ^B (2062)	6557 ^c (1730)	6785 ^{BC} (1755)	< 0.001
305 FCM (kg)	min. – max.	2321-13915	2288-13467	2303-13915	2851-11494	~0.001
305 FPCM (kg)	mean (SD)	7916 ^A (2518)	6987 ^B (2182)	6178 ^c (1707)	6338 ^c (1852)	< 0.001
505 FPCIVI (Kg)	min. – max.	2382-13626	2382-14431	2383-12776	2747-11673	~0.001

Table 1. The effect of the fat to protein (F/P) ratio in the first 60 days in milk (DIM) on selected lactation traits

FPCM - fat protein corrected milk; FCM - fat corrected milk; SCC - somatic cell count; SCS - somatic cell score.

The means in rows denoted by different letters differ significantly at P≤0.01.

: 1), the urea level was 198 mg/L, while in the group with the F/P ratio >1.7: 1 group the mean urea level in the first two months of lactation was 186 mg/L.

Table 2 presents the effect of the fat-protein ratio on daily milk yields, taking into account the factors set out in the Material and Methods section, i.e. herd, lactation,

Table 2. The	effect of th	Table 2. The effect of the F/P ratio, herd, lactation and production level on daily milk yield (kg) in the first 60 days lactation	, lactation a	nd production l	evel on dail	y milk yield (kg	g) in the firs	t 60 days lactat	ion	Ĩ
F	1.1	≤1.1-≥1.7 : 1	VI	≤1.1 : 1	F/I 1.1	F/P ratio 1.1-1.4 : 1	1.4	.4-1.7 : 1	≥1.7 :]	:1
Factor	no observation	on mean (SD)	no observation	m mean (SD)	no observation	m mean (SD)	no observation	n mean (SD)	no observation	mean (SD)
Herd										
1	48	$29.4^4(6.8)$	10	$28.9^{5}(6.5)$	22	28.7 ^{4,5} (7.4)	13	31.2 ⁶ (6.1)	ю	32.36 (8.6)
7	141	$39.4^7(8.3)$	51	$42.1^7(8.6)$	61	$38.7^7(8.1)$	18	$36.5^8 (6.70)$	11	34.9^7 (6.8)
ю	281	$24.1^{2}(5.7)$	14	$20.3^{1}(6.9)$	92	$24.7^{3}(5.9)$	131	$24.3^{2,3}(5.5)$	44	$22.9^{2}(5.2)$
4	85	$30.1^{5}(7.1)$	19	$36.1^7(6.6)$	53	$29.1^{5}(6.3)$	11	26.3^4 (6.2)	2	$22.5^{2}(2.4)$
5	69	$22.1^{1}(5.1)$	13	$25.3^3(3.3)$	34	$20.7^{1}(5.1)$	15	$21.1^{1}(5.4)$	7	$23.9^3(5.2)$
9	42	$21.8^{1}(7.0)$	8	$21.4^{2}(8.9)$	13	$23.1^{2}(6.3)$	18	21.2 ¹ (7.2)	ŝ	$20.9^{1}(6.5)$
7	55	$26.9^{3}(6.4)$	10	$29.2^{5}(8.4)$	26	$27.6^{4}(6.9)$	16	$24.8^3(3.5)$	ŝ	$25.7^{4}(4.4)$
8	55	$28.5^4(6.3)$	8	$27.3^{4}(8.8)$	28	$28.4^4(4.8)$	18	$28.9^{5}(7.7)$	-	$30.4^{5}(0.0)$
6	55	$24.1^2(4.9)$	5	$21.2^{2}(7.1)$	21	$24.3^{3}(4.7)$	19	$23.5^{2}(4.8)$	10	25.6^4 (4.6)
10	60	$33.5^{6}(6.7)$	10	$31.9^{6}(7.1)$	32	$34.8^{6}(6.4)$	13	$33.0^{6,\hat{1}}(6.6)$	5	$30.1^{5}(7.7)$
Lactation										
1	306	24.5 ¹ (7.4)	43	$27.2^{1}(9.1)$	132	$25.3^{1}(7.5)$	102	23.1 ¹ (6.2)	29	21.7^{1} (6.8)
7	228	$28.9^{2}(8.8)$	47	$34.7^3(12.5)$	104	$28.3^{2}(7.5)$	58	$25.9^{2}(5.9)$		27.9^3 (4.8)
С	155	$30.2^{2}(7.8)$	23	$38.1^4(9.2)$	51	$31.2^{3}(6.9)$	61	$27.8^{3}(6.1)$	20	$25.8^{2}(6.1)$
4-9	202	$31.3^3(9.2)$	35	$33.3^{2}(9.7)$	95	$32.4^4(9.5)$	51	$28.8^{4}(8.7)$		28.8^3 (6.8)
Production										
10001 (AB) <20	158	17.2 ¹ (2.2)	22	$17.1^{1}(3.1)$	57	$17.3^{1}(2.1)$	60	17.4 ¹ (1.8)	19	$16.7^{1}(2.1)$
20-30	425	$25.2^{2}(2.8)$	4	$25.6^{2}(3.1)$	176	$25.1^{2}(2.9)$	152	$25.1^{2}(2.7)$		$25.6^{2}(2.7)$
>30	308	$37.8^3(6.3)$	82	$40.7^{3}(7.3)$	149	$37.2^{3}(5.7)$	60	36.1^3 (4.9)		$35.4^3(5.7)$
Total	891	28.2 (8.7)	148	$32.7^{A}(11.1)$	382	$28.6^{B}(8.4)$	272	$25.8^{\rm C}(7.0)$	89	25.6 ^C (6.8)
The means ir The means ir	columns w row denote	The means in columns within factors denoted by different numbers differ significantly at P≤0.01 The means in row denoted by different letters differ significantly at P≤0.01.	noted by dif etters differ	ferent numbers significantly at	differ signif P≤0.01.	ïcantly at P≤0.(01.			

and production level. It was observed that the production level was a key element in reducing milk yields in different F/P ratio groups indicating different ketosis risks. In the herds the animals with the highest production level of 30 kg responded to the increased F/P ratio with a particular reduction in the daily milk yield. In this group of cows an increase in the fat-protein ratio from 1.1 : 1 to >1.7 : 1 decreased daily milk yield dramatically by 7.3 kg (22%) from 32.7 kg to 25.6 kg. The differences were statistically significant at P \leq 0.01.

Statistically significant correlation coefficients and linear regression equations for the fat-protein ratio and milk yield and quality are given in Table 3 and 4. Correlation coefficients for the F/P ratio, daily milk yield (kg), 305-day milk yield (kg), the content of fat, protein, dry matter, and lactose (%), and urea level in milk (mg/L) were as follows: -0.25 (p<0.001), -0.25 (p<0.001), 0.86 (p<0.001), -0.18 (p<0.001), 0.63 (p<0.001), -0.09 (p<0.001), and -0.11 (p<0.001).

Correlated traits	F/P ratio
305 day milk yield (kg)	-0.25 (<0.001)
Daily milk yield (kg)	-0.25 (<0.001)
Fat content in milk (%)	0.86 (<0.001)
Protein content in milk (%)	-0.18 (<0.001)
Dry matter content in milk (%)	0.63 (<0.001)
Lactose content in milk (%)	-0.09 (<0.001)
Urea in milk (mg/L)	-0.11 (<0.001)
SCS in milk	0.05 (0.024)

 Table 3. Pearson correlation coefficients for the F/P ratio and selected milk traits

Table 4. Regression equations for the F/P ratio and selected milk traits

Correlated traits	Regression equation $(y = a + b \times x)$	P-value
F/P ratio value and 305 day milk yield (kg)	$y = 9224 - 1778 \times x$	< 0.001
F/P ratio value and daily milk yield (kg)	$y = 38.4 - 7.61 \times x$	< 0.001
F/P ratio value and fat content in milk (%)	$y = 0.42 + 2.77 \times x$	< 0.001
F/P ratio value and protein content in milk (%)	$y = 3.41 - 0.23 \times x$	< 0.001
F/P ratio value and dry matter content in milk (%)	$y = 9.40 + 2.51 \times x$	< 0.001
F/P ratio value and lactose content in milk (%)	$y = 4.91 - 0.08 \times x$	< 0.001
F/P ratio value and urea in milk (mg/L)	$y = 234.9 - 30.4 \times x$	< 0.001
F/P ratio value and SCS in milk	$y = 4.53 + 0.23 \times x$	0.024

The negative highly significant correlation coefficients obtained in the study between the size of the F/P ratio and milk yield (daily and lactation), protein and lactose contents indicate that in the population of dairy cows the occurrence of ketosis leads to a reduction in the milk yield of cows. The study also found a radical increase in the level of fat in cow's milk with an increase in the F/P ratio. The intensity of the daily milk yield loss due to the F/P ratio resulted in the severity of the F/P ratio increase in milk. Each additional 0.2 increase in the F/P ratio (beyond 1.0:1) led to a 1.52 kg daily milk loss for the first 60 DIM and 352 kg for 305 DIM. Similarly, each 0.2 increase in the F/P ratio resulted in an increase in fat content by 0.63% and a decrease in protein and lactose contents by 0.05 and 0.02%, respectively, in the first 60 DIM.

The effect of SCK on milk production was described by several authors. Its effect on a milk yield decrease was studied using data from 23,416 Finnish Ayrshire cows [Rajala-Schultz *et al.* 1999]. The daily milk loss was greatest within 2 weeks after the diagnosis, varying from 3.0 to 5.3 kg/day, depending on parity. Cows in parity 4 or higher were most severely affected by ketosis; the average total loss per cow was 353.4 kg. Subclinical ketosis during the first week of lactation can decrease milk yields by up to 2.48 kg a day, while cows affected by the disease are on average three times more likely to be removed from the herd [Oetzel 2007]. In the USA for every 0.1 mmol/L increase in β HBA levels beyond 1.2 mmol/L, a 0.59 kg milk loss was recorded [Oetzel 2012].

A 2.21 kg decrease (by about 5.5%) in the milk yield for cows with blood β HBA \geq 1.4 mmol/L during the first week after calving was reported by Duffield *et al.* [2009]. Using a cutoff point of ≥ 1.0 mmol/L of blood β HBA to define SCK, Ospina *et al.* [2010a] found that cows (*Elactation 2*) with SCK lost 467 kg of 305-day ME (mature equivalent) milk (about 7.0%). Cows in 32 southern Ontario Holstein herds were monitored by Dohoo and Martin [1984] for subclinical ketosis for two and a half years. Milk ketone scores of +1 and +2 were accompanied by a reduction in daily milk production of 1.0 and 1.4 kg, respectively. Chapinal et al. [2012] reported a 2.8 kg reduction in milk yield (about 6.9%) at the first Dairy Herd Improvement Association test for cows with blood β HBA \geq 1.4 mmol/L during the first week after calving. In turn, first-parity cows of the Holstein breed were studied by Gantner et al. [2016] in Croatia and a significant negative effect of subclinical ketosis on the daily milk yield for each parity was found. A decrease in the milk yield of 4.21, 2.73, 2.78 and 2.83 kg/day in each parity (i.e., parities 1, 2, 3 and 4+) was recorded within 35 days after the detection of subclinical ketosis. The daily milk yield decreased within 35 days after the subclinical acidosis detection date, with a loss of 1.4, 1.1, 2.79, and 1.74 kg of milk per day in parity class 1, 2, 3 and 4+. According to McArt et al. [2012], cows (any parity) with SCK produced 1.4 kg less daily milk (about 3.4%) for the first 30 days in milk (DIM) compared to non-ketosis cows.

In conclusion of their investigation, Yang *et al.* [2019] stated that feed intake lower than that required to maintain high milk production in the early postpartum period is assumed to cause ketosis in dairy cows. High plasma concentrations of β HBA and nonesterified fatty acids (NEFA), high milk fat and citrate levels (the latter considered an early indicator), and lower milk protein and lactose concentrations are symptoms of ketosis. Average levels of milk fat, milk protein and lactose of affected cows were as follows: 4.43, 2.78 and 4.91%. The purpose of a study by Vince *et al.* [2017] was to assess the effects of SCK prevalence on the risk of some periparturient diseases and drop in milk production. The studies were conducted in 107 randomly selected farms on 841 cows aged 2-8 years, divided into two groups: positive, with clinical or subclinical ketosis, and a negative (control) group. Contrary to the results obtained in own research, Vince *et al.* [2017] concluded that the positive group of cows had a significantly higher mean lactation yield than the negative group of cows (7076 kg vs. 6409 kg).

A milk yield loss due to SCK was accompanied by an elevation in β HBA at the first diagnosis of the disease [McArt *et al.* 2012]. Each additional 0.1 mmol/L increase in β HBA (beyond 1.2 mmol/L) resulted in 0.59 kg of lost milk for the first 30 DIM. The difference between moderate SCK (1.2 mmol/L β HBA) and more severe SCK (2.4 mmol/L) was 7.1 kg of daily milk for the first 30 DIM.

Vlček *et al.* [2016] studied 680 records (from 3 to 150 days) of 188 cows from 4 farms in western Slovakia between 2013 and 2016. The highest prevalence of ketosis risk (19.08%) was found in the first month after calving. In this group of cows the average milk yield was 35.83 kg per day. Due to the effect of the fat-to-protein ratio a decrease in milk yield was 1.2 kg per day.

In the present study a negative energy balance manifested by an increase in the F/P ratio resulted in a decrease in the yield of milk and in significant changes in its basic chemical composition. An increase in the F/P ratio from 1.1-1.4:1 to >1.7:1decreased the daily milk yield by 3 kg, protein and lactose percentage by 0.16 and 0.09%, respectively, and 305-day milk yield by 649 kg. The severity of the daily milk yield loss due to the F/P ratio was associated with the magnitude of the increase in the F/P ratio in milk. Each additional 0.2 increase in the F/P ratio (beyond 1.0 : 1) resulted in a 1.52 kg loss of daily milk for the first 60 DIM and 352 kg for 305 DIM. Similarly, each 0.2 increase in the F/P ratio increased milk fat content by 0.63% and decreased protein and lactose contents by 0.05 and 0.02%, respectively, in the first 60 DIM. In addition, a significant increase in the number of somatic cells in the milk of cows with the fat-protein ratio >1.7:1 was recorded. On average, milk from this group contained 253,000/L somatic cells compared to milk with the optimal fat-protein ratio (1.1-1.4:1). The results of the study show that the occurrence of ketosis in dairy herds leads to significant changes in the chemical composition of milk. Another effect is the reduction of milk yield in lactation.

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