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ORIGINAL PAPER

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THE EFFECT OF DIFFERENT CONTENT OF PROTEIN AND UREA IN MILK, AS BIOMARKERS OF ENERGY-PROTEIN BALANCE OF FOOD RATIONS, ON THE LEVEL OF SELECTED MILK PERFORMANCE CHARACTERISTICS OF POLISH HOLSTEIN-FRIESIAN COWS

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ABSTRACT

The aim of the study was to assess the impact of varied levels of milk protein and urea content on selected milk production traits of Polish Holstein-Friesian cows. The research included 34,397 data sets related to daily milk production of Polish Holstein-Friesian cows. These animals were kept in 15 cattle herds located in the Mazovian and Podlasie voivodeships. In the first stage of the research, the animals were differentiated based on the level of urea in their milk and the percentage of protein. According to the adopted assumptions, the energy and protein balance level of feed rations was determined based on the concentration of urea and protein in the milk. Due to the level of urea, three cow groups were distinguished: <150, 150–250, and >250 mg $\cdot$ L^{-1}. Depending on the concentration of protein in the milk, the following three groups of animals were determined: <3.2%, 3.2–3.6%, and >3.6%. The assessment of the impact of varied energy and protein balance levels in feed rations on selected milk production traits of cows was conducted in subsequent lactation periods, production level and fat to protein (F/P) groups. Four lactation periods were distinguished, which covered successive months of lactation: 1–3, 4–6, 7–10, and 11–18, three production groups, determined based on the following levels of daily milk yield: <20, 20–30, and >30 kg and as well three groups of cows with different F/P ratio in milk were distinguished: <1.2, 1.2–1.6, >1.6. In the next stage of the study, a detailed assessment was conducted on the impact of varied levels of energy and protein balance in feed rations on the analyzed milk production traits. The analyzed population of cows was divided into 9 groups based on the level of urea content (mg $\cdot$ L^{-1}) and protein concentration (%), with varying levels of coverage of the protein and energy requirements of cows. The interdependencies between milk performance traits of cows fed diets with balanced levels of energy and protein and their peers, for whom the nutritional diets were differentiated based on energy-protein balance, were determined using Pearson correlation coefficients and linear regression. When assessing the energy supply of cows, the study showed that too low energy levels in diets lead to a decrease in the chemical composition of milk, while too high levels lead to a decrease in milk yield compared to peers fed optimally balanced diets. Milk from cows with inadequate energy and protein nutrition contained less fat and protein by 0.54% and 0.59%, respectively, compared to milk from cows fed optimally. On the other hand, excessive energy supply was accompanied by an increase in fat (+0.66%) and protein (+0.57%) concentration with a decrease in daily milk yield (–4.1 kg). When evaluating the level of protein coverage for cows, the study showed that an excess of protein in feed led to an increase in urea levels in milk. The level of urea in milk from cows fed with excess protein in their feed (>250 mg $\cdot$ L^{-1} and <3.2%; >250 mg $\cdot$ L^{-1} and >3.6%; >250 mg $\cdot$ L^{-1} and 3.2–3.6%) contained more urea per liter compared to milk from cows fed a balanced diet, with increases of 127, 107, and 109 mg, respectively.

Key words: dairy cows, feed ration, balance, milk traits

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INTRODUCTION

The chemical composition and unit yield of cow's milk are not constant. Rational nutrition, along with their genetic potential, are two key factors determining the level of these traits in dairy cattle. Feeds used in cattle feeding provide nutrients that are directly or indirectly transformed into milk and its chemical components.

Milk fat is the most susceptible among all milk components to changes in concentration caused by animal nutrition. According to O'Callaghan et al. [2016], Micek et al. [2019] and Daley et al. [2022] the variability of fat concentration in cows' milk depends to the greatest extent on the feeding technology. Changes in the content of fat in cow's milk can exceed even 3 percentage points depending on the feeding technology [Looper 2012]. Underfeeding cows, feeding excessive amounts of fibrous feed, as well as excess protein, energy deficiency, and lack of voluminous feed in the ration are among the most common reasons for reducing the level of fat in milk [Lee et al. 2014, Guliński et al. 2018]. Increasing the share of voluminous feed with a high fiber content in the ration, such as hay, silage, good quality silage, or even good straw, lead to the higher the fat content. Dietary fiber provided in feed for cattle is divided into neutral detergent fiber (NDF) and acid detergent fiber (ADF). For dairy cows, NDF is particularly important as it serves as a source of energy for rumen microorganisms, and it also gives structure to the feed and provides bulk that fills the rumen. The daily requirement for ADF is between 19 and 21 percent of the dry matter (DM) intake [NASEM 2021]. The total daily requirement for NDF should not exceed 28–32% DM for cows producing >9000 L, 30–34% DM for late lactation/medium production, 34–40% DM for cows producing <6000 L [Tylutki et al. 2008]. In turner, feeding cows with concentrated feeds with a high content of easily fermentable carbohydrates in the rumen leads to a decrease in rumen pH and subclinical acidosis [Plaizier et al. 2008, Lawrence et al. 2015, Fiorentin et al. 2018] This situation is associated with a reduction in the percentage of fat in milk, which in extreme cases can take the form of fat depression [Krause and Oetzel 2006, Rivero and Anrique 2015].

Inducing changes in milk protein content through nutrition is much more limited compared to changes in milk fat content, mainly due to lower genetic variability in protein content [Guliński et al. 2018]. Dietary manipulation results in milk protein concentration changing approximately 0.60 percentage units [Looper 2012]. Milk protein content depends mainly on the amount of energy supplied in the diet, with energy deficiency usually leading to a decrease in its level [Vanbergue et al. 2018, Herve et al. 2019, Leduc et al. 2021]. However, a high proportion of concentrated feed in the diet, which increases the amount of readily digestible energy, feeding high-protein feeds (including protected protein), providing cows with amino acids protected from degradation in the rumen (mainly methionine and lysine), and a low ratio of roughage to concentrated feed can increase the level of protein in milk [McAuliffe et al. 2016, Gulati et al. 2018; Panthi et al. 2019]. Khan et al. [2015] showed that cattle ration supplemented with maize silage is the optimal option for high protein content in milk. Also in the studies of Dalley et al. [2020], supplementation of food rations with maize silage resulted in a high percentage of protein in milk (3.99%). One of the reasons for the decrease in the percentage of protein in milk may be too high a level of protein in the rations. According to Leonardi et al. [2003] the increase in the share of protein in the dry matter of the ration from 16.1% to 18.8% was associated with a decrease in the percentage of protein in milk from 3.24 to 3.18%. According to Colmenero and Broderick [2006], the optimal level of protein in the dry matter of the ration for dairy cows is 16.5%. In the opinion of these authors, such a level of protein is optimal from the point of view of the level of protein in milk and the optimization of the level of urea excreted in urine and in milk.

The most stable component of cow's milk is lactose. This is due to the fact that there is a close correlation between the synthesis of lactose and the amount of water taken into milk. In production conditions, changes in lactose levels occur only as a result of lowering the hygienic quality of milk and increasing the number of somatic cells [Costa et al. 2019, Alessio et al. 2021]. Lactose is easily metabolized by microorganisms, which makes milk one of the easily fermented substances. The results of a number of studies also indicate the age of cows as a factor determining the level of lactose in milk [Haile-Mariam and Pryce 2017, Costa et al. 2019]. In studies of Australian dairy cattle and Italian Holsteins, these authors showed a decrease in lactose levels with the age of the cows.

The presence of urea in milk is the result of protein transformations in cows' organisms. As a result, this chemical compound appears in the body fluids. The results of many available domestic and foreign studies show high variability in the level of this milk component [Lavery and Ferris 2021]. Changes in its level in milk are primarily influenced by the level of protein in feed rations. According to Jonker et al. [1999] and Kebreab et al. [2002], an increase in the share of true protein in dry matter dose from 13 to 18% leads to an increase in the concentration of urea in milk from 70 to over 150 mg in 1 liter of milk. Rations rich in minerals, such as sodium (Na), potassium (K), lead to increased water consumption and increased urine production, thereby reducing the level of urea in milk and blood plasma [Spek et al. 2012]. In research by Spek [2013], an increase in sodium intake from 69 to 419 g per day was accompanied by an increase in water intake (from 61.7 to 115.7 kg), an increase in the volume of urine produced by a cow (from 18.2 to 67.7 kg per day) and a decrease in the level of urea in milk (from 152 to 118 mg $\cdot$ L^{-1}). Other factors influencing the level of urea in milk also include: type and technology of feed administration [Guliński et al. 2016], number of milkings and length of break between milkings [Nielsen et al. 2005], body weight [Zhai et al. 2007], water consumption [Burgos et al. 2001, Spek 2013].

Modern knowledge provides practical possibilities for assessing the impact of balancing the energy and protein needs of cows on their milk production characteristics. One of the basic tools in this area is monitoring the level of urea in milk, which allows for an assessment of the protein content in the diet. In Poland, the optimal level of urea in raw milk from cows reflecting their protein needs balance is considered to be between 150–250 mg per liter. A level that is too low (<150 mg $\cdot$ L^{-1}) or too high (>250 mg $\cdot$ L^{-1}) indicates an imbalance in protein needs and suggests that the feed contains too low or too high a level of protein in relation to energy [Nousiainen et al. 2004, Roy et al. 2011, Bostanovaa et al. 2022]. When evaluating and interpreting the level of urea in milk, the level of protein in the milk is also taken into account. There is a close correlation between these compounds, as well as between the energy-protein balance in feed rations [Sawa et al. 2010]. Although the protein content in milk is largely regulated by the animals' genotype, changes in its level can also be a result of nutritional deficiencies. The consequence of too much energy supply in feed rations is an increased protein concentration, and too little leads to a decreased level of protein. The correct protein content for Polish Holstein-Friesian cows, reflecting optimal energy requirements, is considered to be in the range of 3.2–3.6%; a level below 3.2% is interpreted as a possible energy deficiency in the feed ration, and a level above 3.6% is assessed as an excess of energy in the applied feed rations [Ziemiński and Juszczak 1997, Ljoljić et al. 2023].

The aim of the study was to assess the impact of varying levels of milk protein and urea content, as biomarkers of energy-protein balance of food rations, on the level of selected milk performance characteristics of Polish Holstein-Friesian cow.

MATERIAL AND METHODS

34,397 data on daily milk yield of Polish Holstein-Friesian cows were included in the study. These animals were kept in 15 cattle herds located in the Mazovian and Podlaskie Voivodeships. The average herd size was 78 cows and ranged from 23 to 170 animals. Animals were kept in 11 herds in the free-stall system and in 4 herds in the station system. The average actual milk yield in a 305-day lactation was 7353 kg and ranged from 5320 (herd 1) to 9676 kg (herd 15). In the feeding of cows in individual herds, the TMR (Total Mixed Ration) and PMR (Partly Mixed Ration) technologies were used. In the first stage of the study, the animals were differentiated based on the level of urea in the milk and the percentage of protein content. Based on the level of urea, the following three groups of cows were distinguished: <150, 150–250, and >250 mg $\cdot$ L^{-1}. Depending on the protein concentration in the milk, the following 3 groups of animals were identified: <3.2%, 3.2–3.6%, and >3.6%. According to the adopted assumptions, the level of energy and protein balance of feed doses was determined based on the concentration of urea and protein in milk. An evaluation of the effect of cows groups for which the levels of milk protein and urea indicate a varied level of energy and protein balance on selected milk production traits of cows was conducted in successive lactation periods, individual production groups and groups of cows with different level fat/protein (F/P) ratio in milk. Four lactation periods were distinguished, which included consecutive months of lactation: 1–3, 4–6, 7–10, and 11–18, three production groups, determined based on the following levels of daily milk yield: <20, 20–30, and >30 kg and additionally, three groups of cows with different F/P ratio in milk were distinguished: $\leq$ 1.2, 1.2–1.6, >1.6 . In the first stage of the study, the impact of energy and protein balance on the following milk production traits was evaluated: actual milk yield (kg), FCM milk yield (kg), percentage of fat and protein in milk, and urea concentration (mg $\cdot$ L^{-1}). Detailed data regarding the number of animals in each individual group are presented in Table 1.

In the second stage of the study, a detailed evaluation of the impact groups of cows with varied levels of milk protein and urea, indicated different of energy and protein balance of feed doses, on the analyzed milk production traits was conducted. For this purpose, the evaluated population of cows was divided into 9 groups based on the level of urea concentration (mg $\cdot$ L^{-1}) and protein concentration (%), where the level of these traits was respectively: I, <150 and <3.2; II, <150 and >3.6; III, <150 and 3.2–3.6; IV, >250 and <3.2; V, >250 and >3.6; VI, >250 and 3.2–3.6; VII, 150–250 and <3.2; VIII, 150–250 and >3.6. The optimal group of cows, whose milk chemical composition indicated optimal energy-protein balance of feed rations, was considered to be the one whose milk protein and urea content was between 3.2–3.6 and 150–250 mg $\cdot$ L^{-1}, respectively (IX group).

A linear model containing effects of level of urea in milk and protein concentration group (feeding group), lactation period, production level group, urea concentration class, and protein in milk group class was used:

$$y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + G_m + F_n + \\ + H_o + (AC)_{ik} + (AH)_{io} + e_{ijklmno}$$

where:

y_{ijklmn} – trait level;
μ – general mean;
A_i – fixed effect of the different level of urea in milk and protein concentration group and feeding group ($i = 1..9$);
B_j – fixed effect of the lactation period group ($j = 1, 2, 3, 4$);
C_k – fixed effect of production level group ($k = 1, 2, 3$);
D_l – fixed effect of the urea content group ($l = 1, 2, 3$);
G_m – fixed effect of protein content group ($m = 1, 2, 3$);
H_o – fixed effect of F/P ratio group ($o = 1, 2, 3$)
F_n – random effect of the cow;
$(AC)_{ik}$ – interaction: feeding group × production level group;
$(AH)_{io}$ – interaction: feeding group × F/P ratio group;
$e_{ijklmno}$ – random error.

The interrelationships between milk production traits in cows fed with balanced levels of energy and protein and their peers, who were fed with varied levels of energy and protein balance, were determined using Pearson correlation coefficients and linear regression. Regression equations were calculated to relate the selected milk production traits, e.g. daily milk yield (kg), daily FCM milk yield (kg), fat (%), protein (%) and urea ($\mathrm{mg \cdot L^{-1}}$). Linear regression coefficients of dependent variables (daily milk yield, daily FCM milk yield, fat, protein and urea) on the a group with a varied level of energy and protein balance in the food ration 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 and Gabriela test at $P \leq 0.01$. The computations were performed using the GLM, FREQ and REG procedures of the SAS statistical package [SAS Institute 2008].

RESULTS AND DISCUSSION

In Table 1, the results concerning the influence of the factors determined in the methodology on the selected milk production traits in the evaluated population of cows are presented. The data in this table indicate that all of the factors determined in the methodology, namely lactation stage, production level, feeding group, and energy and protein balance level in the feed rations and F/P ratio groups significantly differentiated the milk production traits of the cows. Evaluating the daily milk yield, it was shown that the highest level of this trait was observed in animals in the first 100 days of lactation and fed with food rations with a too low level of energy. Cows belonging to these groups produced 27.8 and 27.3 kg of milk, respectively. The highest daily milk yield demonstrated in the study within the highest production level (36.4 kg) should be considered as an obvious result resulting from the criteria for dividing animals into production levels adopted in the study.

Table 1 also provides an assessment of the percentage share of milk samples analyzed in the study, taking into account the variability factors. The data showed a large variation in the assessed population in terms of the level of energy and protein balance in the feed rations used in cow feeding. It was found that the optimal level of energy or protein in the rations was characterized by 35% and 56.3% of all observations, respectively. Too low levels of energy and protein in the feed rations were observed in 31.5% and 22.8% of milk samples, respectively. The data on the share of particular feed groups in the entire population also indicate significant problems with the optimal balancing of cow's nutritional needs. Only 5.2% of observations indicated full energy and protein balance. The most commonly occurring were rations with an optimal level of protein and a too low or too high proportion of energy. The share of such identified feed groups was 24.6% and 27.3%, respectively.

The analysis of the level of two basic chemical components of milk showed that with the increase in the length of the postpartum period and the increase in the level of energy in the feed, there was a systematic increase in the percentage content of these milk components. Thus, in the analyzed population, the average concentration of fat and protein between the first and last trimester of lactation increased by 0.83% and 0.89%, respectively. Similarly high differences were observed in the fat and protein content in the milk of cows fed diets with either too high or too low level of balanced energy requirements. The differences in the concentration of fat and protein in the milk between these groups of cows were 0.99% and 0.99%, respectively. The conducted analysis of variance confirmed the significance of these differences with $P \leq 0.01$.

Discussing the influence of a nutrition group on the development of milk production traits, the hypothesis was adopted that for the evaluated population of Polish Holstein-Friesian cows, the level of milk production traits characteristic of group IX animals should be considered typical for the breed. This group comprised animals whose feed doses were fully balanced in terms of protein and energy requirements. The results obtained for this group of cows showed that they produced an average of 23.5 kg of milk per day, and their milk contained on average 4.19% fat, 3.40% protein, and 200 mg of urea per liter. The values of milk production traits obtained for the other nutrition groups were highly varied. This fact indicates that in the population of dairy cattle, providing animals with feed rations with varying levels of balance

Table 1. The number of observation and effect of the analyzed factors on the selected milk performance traits

Factors	Observation		Milk traits				
			Actual milk, kg	FCM milk, kg	Fat, %	Protein, %	Urea, mg · L^{-1}
	N	%	x̄ ±SD	x̄ ±SD	x̄ ±SD	x̄ ±SD	x̄ ±SD
Production level (kg per day)							
<20	14915	43.4	14.4^{C} ±4.0	15.6^{C} ±4.8	4.52^{A}±1.07	3.58^{A}±0.69	197^{C} ±107
20–30	12438	36.2	24.8^{B} ±2.9	25.7^{B} ±3.8	4.26^{B}±0.75	3.39^{B}±0.41	205^{B} ±74
>30	7044	20.4	36.4^{A} ±5.3	35.2^{A} ±5.8	3.81^{C}±0.73	3.19^{C}±0.32	208^{A} ±75
Lactation period (months)							
1–3	9641	28.1	27.8^{A} ±10.3	27.7^{A} ±10.1	3.95^{D}±1.06	3.03^{D} ±0.61	198^{C} ±74
4–6	9635	28.0	23.8^{B} ±8.0	24.0^{B} ±7.6	4.12^{C}±0.77	3.36^{C} ±0.35	209^{A}±124
7–10	11037	32.1	19.4^{C} ±9.1	20.6^{C} ±7.1	4.51^{B}±0.84	3.65^{B} ±0.41	203^{B} ±76
11–18	4084	11.8	16.0^{D} ±6.7	17.7^{D} ±7.0	4.78^{A} ±0.86	3.92^{A} ±0.44	195^{D} ±65
Groups of cows with different milk urea and protein content (level of protein and energy supply in feed rations)							
I	1150	3.3	24.6^{C}±10.1	23.7^{D} ±9.9	3.65^{E}±1.07	2.81^{E}±0.63	106^{E} ±33
II	1032	3.0	16.0^{H} ±6.9	17.9^{H} ±7.5	4.86^{A}±0.87	3.99^{A}±0.33	108^{E}±31
III	5416	15.8	21.7^{E} ±8.2	22.1^{E} ±8.1	4.19^{B}±0.75	3.39^{C} ±0.12	107^{E} ±31
IV	965	2.8	25.9^{B} ±9.5	25.2^{B} ±9.1	3.83^{C}±0.90	2.90^{D}±0.44	322^{A} ±32
V	1103	3.2	19.2^{F} ±8.0	21.4^{F} ±8.8	4.86^{A} ±0.81	3.99^{AB} ±0.32	305^{C} ±50
VI	5125	14.9	24.4^{C} ±9.1	24.7^{C} ±9.0	4.16^{B}±0.72	3.40^{C} ±0.12	310^{B} ±57
VII	8430	24.6	27.0^{A} ±9.8	26.2^{A} ±9.4	3.76^{D} ±0.91	2.89^{D} ±0.49	197^{D} ±28
VIII	9395	27.3	18.3^{G} ±7.1	20.5^{G} ±7.7	4.87^{A}±0.81	3.97^{B} ±0.31	200^{D} ±28
IX	1781	5.2	23.5^{D} ±8.2	23.9^{D} ±8.0	4.19^{B} ±0.68	3.40^{C} ±0.11	200^{D} ±28
Groups of cows with different level milk protein content (level of energy supply in feed rations)							
<3.2%	10831	31.5	27.3^{A}±9.1	26.4^{A}±8.6	3.86^{C}±0.70	2.97^{C}±0.17	200^{B}±119
>3.6%	11530	33.5	18.3^{C}±7.2	20.5^{C}±7.8	4.85^{A}±0.81	3.96^{A}±0.24	203^{AB}±52
3.2–3.6%	12036	35.0	23.1^{B}±8.6	23.5^{B}±8.6	4.19^{B}±0.73	3.40^{B}±0.11	205^{A}±102
Groups of cows with different level of milk urea content (level of protein supply in feed rations)							
<150 mg · L^{-1}	7847	22.8	21.6^{C}±8.6	22.1^{C}±8.4	4.23^{B}±0.80	3.41^{C}±0.33	109^{C}±31
>250 mg · L^{-1}	7222	20.9	23.9^{A}±9.2	24.4^{A}±9.1	4.23^{B}±0.80	3.43^{B}±0.32	312^{A}±139
150–250 mg · L^{-1}	19328	56.3	22.8^{B}±9.2	23.7^{B}±8.6	4.36^{A}±0.89	3.48^{A}±0.53	200^{B}±28
Groups of cows with different level Fat/Protein ratio in milk							
≤1.2	14074	40.9	24.1^{A}	22.8^{C}	3.71^{C}	3.49^{A}	204^{A}
1.2–1.6	18369	53.4	21.5^{C}	23.2^{B}	4.59^{B}	3.42^{B}	202^{AB}
>1.6	1954	5.7	22.1^{B}	26.8^{A}	5.26^{A}	2.96^{C}	198^{B}
Total / average	34397	100	22.5 ±9.3	23.1 ±8.9	4.28 ±0.24	3.43 ±0.56	202 ±90

The means in the columns within the factors marked with different letters differ significantly at $P \leq 0.05$.

in their energy and protein requirements is one of the fundamental factors contributing to the variability of traits related to both milk yield and its chemical composition.

Table 2 present data on the effect of the varied content of urea and protein in milk, as biomarkers of protein-energy balance of food rations, on the analyzed milk performance characteristics.

The results of this table indicate a high and statistically confirmed effect of the varied content of protein and urea in milk on the milk performance characteristics of cows assessed in the study. In general, in the analyzed

Table 2. The effect of the varied content of urea and protein in milk as biomarkers of protein–energy balance of food rations on the analyzed milk performance characteristics

Factors	Differences in the values of traits in relation to the mean for the optimal group				
	Actual milk, kg	FCM milk, kg	Fat, %	Protein, %	Urea, mg · L^{-1}
Groups of cows with different milk urea and protein content (level of protein and energy supply of feed rations)					
I	+1.1*	–0.9	–0.54*	–0.59*	–94*
II	–7.3*	–5.5*	+0.66*	+0.57*	–92*
III	–1.7*	–1.8*	+0.01	–0.01	–93*
IV	+2.3*	+0.5	–0.35*	–0.51*	+127*
V	–4.1*	–1.8*	+0.66*	+0.57*	+107*
VI	+0.9	+0.8	–0.03	–0.01	+109*
VII	+5.3*	+1.7*	–0.42*	–0.51*	–3
VIII	–5.1*	–2.9*	+0.66*	+0.55*	+1
IX x̄ (optimal group)	23.5	23.9	4.19	3.40	200
Groups of cows with different level milk protein content (level of energy supply of feed rations)					
1 – <3.2% (too low)	+3.6*	+2.3*	–0.42*	–0.51*	–5
3 – >3.6% (too high)	–4.7*	–3.1*	+0.66*	+0.56*	–2
2 – 3.2–3.6% x̄ (optimal group)	23.1	23.5	4.19	3.40	205
Groups of cows with different level of milk urea content (level of protein supply of feed rations)					
1 – <150 mg · L^{-1} (too low)	–1.2	–1.5	–0.09	–0.05	–91*
3 – >250 mg · L^{-1} (to high)	+1.1	+0.9	–0.11	–0.02	+112*
2 – 150–250 mg · L^{-1} x̄ (optimal group)	22.8	23.7	4.36	3.48	200

* Difference in the values of particular trait compared to the mean traits for the optimal group significant at $P \leq 0.05$.

population of Holstein-Friesian cows, administration of high-energy rations was associated with an increase in the percentage of protein in milk by +0.56% compared to the level of protein in the milk of optimally fed cows. The increase in protein concentration in milk associated with the high level of energy in the doses concerned all groups of animals, regardless of the protein level in the doses, which was manifested by the varying levels of urea in the milk. The inverse relationship was related to milk obtained from animals fed with deficient doses in terms of energy levels. In the whole assessed population, the milk of these animals contained –0.51% less protein compared to optimally fed animals.

As in the case of energy, the study showed a high and statistically confirmed effect of the level of protein balance of food rations on the level of urea in milk. The excess level of protein in the rations was accompanied by a radical increase in the level of urea, which on average for the entire cow population was +112 mg in 1 liter of milk. The data in Table 2 indicate that this increase occurred in all animals at the doses with excess protein levels, regardless of the level of energy balance of these doses. It should also be emphasized that the high level of energy in the doses for this group of cows mitigated the effects of excess protein in the doses, consequently leading to a decrease in the level of urea in the milk. Its level in the milk of cows containing more than 250 mg of urea and more than 3.6% of protein was lower by 20 mg · L^{-1} compared to animals whose milk contained more than 250 mg of urea and more than 3.6% of protein.

Milk from cows with energy and protein deficient diets (<3.2% and <150 mg · L^{-1}) contained less fat and protein compared to milk obtained from cows fed optimally, by 0.54% and 0.59%, respectively. Conversely, excess energy feeding (>3.6% and <150 mg · L^{-1}) was accompanied by an increase in fat concentration (+0.66%) and protein (+0.57%) with a decrease in daily milk yield (–7.3 kg). Regarding the level of protein coverage for cows, the study showed that excess protein in the feed rations led to an increase in urea levels in the milk. The level of urea in the milk of cows fed with excess protein in their feed rations (>250 mg · L^{-1} and <3.2%; >250 mg · L^{-1} and >3.6%; >250 mg · L^{-1} and 3.2–3.6%) contained more urea per liter compared to milk from cows fed rationally, with increases of 122, 107, and 109 mg, respectively

The data presented in Table 2 provide grounds to conclude that in production conditions the energy-protein

Table 3. The effect of groups of cows with different milk protein and urea content, and the level of cow production on the analyzed milk performance characteristics

Production level, kg per day	Milk traits	Groups of cows with different milk urea (mg · L⁻¹) and protein content (%) (level of protein and energy supply of feed rations)								IX (optimal)
		I	II	III	IV	V	VI	VII	VIII	
		Differences in the values of traits in relation to the mean for the optimal group								x̄
≤20	Actual milk, kg	–1.2*	–2.4*	–0.5	–0.5	–1.5*	–0.5	–0.5	–1.1*	14.4
	FCM milk, kg	–2.6*	–1.4*	–0.6	–1.3*	–0.4	–0.7*	–1.5*	+0.1	15.6
	Fat, %	–1.1*	+0.58*	–0.03	–0.51*	+0.63*	–0.08	–0.77*	+0.63*	4.52
	Protein, %	–0.84*	+0.60*	–0.01	–0.66*	+0.61*	–0.01	–0.74*	+0.58*	3.58
	Urea, mg · L⁻¹	–97*	–90*	–92*	+173*	+114*	+120*	–3	+1	197
20–30	Actual milk, kg	+0.1	–1*	–0.4	+0.4	–0.3	+0.1	+0.3	–0.5*	24.8
	FCM milk, kg	–0.8*	+0.4	–0.4	–0.4	+1.7*	–0.2	–0.4	+1.1*	25.7
	Fat, %	–0.25*	+0.39*	–0.01	–0.27*	+0.54*	–0.01	–0.23*	+0.46*	4.26
	Protein, %	–0.46*	+0.46*	–0.02	–0.46*	+0.51*	–0.01	–0.45*	+0.47*	3.39
	Urea, mg · L⁻¹	–93*	–92*	–93*	+109*	+98*	+102*	–4	+1	205
>30	Actual milk, kg	+2.0*	–0.5	–0.4	+1.6*	+0.5	+1.5*	+2.3*	–0.5	36.4
	FCM milk, kg	+0.6	+2.4*	–0.5	+0.5	+3.1*	+1.8*	+0.9	+2.1*	35.2
	Fat, %	–0.20*	+0.58*	–0.03	–0.17	+0.45*	+0.07	–0.22*	+0.52*	3.81
	Protein, %	–0.43*	+0.50*	–0.03	–0.41*	+0.49*	–0.01	–0.41*	+0.44*	3.19
	Urea, mg · L⁻¹	–94*	–104*	–96*	+105*	+109*	+106*	–4	+4	208

* Difference in the values of particular trait compared to the mean traits for the optimal feeding group significant at $P \leq 0.05$.

balance of the rations, manifested by the appropriate level of protein and energy, is one of the key factors of variability of the basic components of milk. Differences in the content of fat, protein and urea in the milk of cows fed with a balanced level of energy or protein in relation to the group of cows fed optimally in terms of energy and protein levels were small and in all cases not statistically significant.

Searching for an answer to the question whether the differences in milk performance characteristics shown in Table 2 depended only on the level of balanced feed rations, Tables 3 and 4 summarized the results of the impact of other factors determined in the methodology, i.e. the production level of animals and the fat-protein ratio in milk . The data in these tables showed that the level of balanced rations leads to a significant differentiation of milk performance characteristics of cows belonging to different production groups and characterized by different F/P ratios in milk. In all subgroups of animals distinguished in Tables 3 and 4, the increase in the level of energy and protein in the doses was accompanied by an increase in the level of protein and urea in milk.

To further determine the influence of diverse feeding of cows on the variability of evaluated traits, in the next stage of the study, an assessment was made of the interdependence between milk production of cows with varying levels of coverage of their energy and protein requirements in their feed doses. Table 5 presents correlation and regression coefficients between these traits. Changes in milk production traits were compared to the group characterized by an optimal level of balance in their energy and protein requirements. The data in this table showed that in the evaluated population of Polish Holstein-Friesian cows, the level of protein and energy balance in feed doses caused significant changes in both milk yield and its chemical quality. The study evaluated the energy supply and showed that a too low level of energy in feed led to a decrease in the milk's chemical composition, while a too high level led to a decrease in milk yield compared to cows fed a balanced diet. Milk from cows with an energy and protein deficiency (<3.2% and <150 mg · L^{-1}) contained less fat and protein by 0.54% and 0.59%, respectively, compared to milk obtained from optimally fed cows. Conversely, excess energy intake (>3.6% and <150 mg · L^{-1}) was associated with an increase in fat (+0.65%) and protein (+0.58%) concentration, with a decrease in daily milk yield (–7.3 kg).

When assessing the level of protein coverage for cows, it was observed that an excess of protein in feed led to an increase in the level of urea. The level of urea in the milk of cows fed with excess protein in their feed (>250 mg · L^{-1} and <3.2%; >250 250 mg · L^{-1} and

Table 4. The effect of groups of cows with different milk protein and urea content and the F/P ratio on the analyzed milk performance characteristics

F/P ratio	Milk traits	Groups of cows with different milk urea ($mg \cdot L^{-1}$) and protein content (%) (level of protein and energy supply of feed rations)								IX (optimal)
		I	II	III	IV	V	VI	VII	VIII	
		Differences in the values of traits in relation to the mean for the optimal group								$\bar{x}$
<1.2	Actual milk, kg	+1.3	–8.8*	–1.9*	+3.1*	–4.9*	+0.6	+4.6*	–5.8*	24.1
	FCM milk, kg	–0.8	–7.1*	–2.1*	+0.9	–2.8*	+0.4	+2.3*	–3.9*	22.8
	Fat, %	–0.55*	+0.54*	–0.08*	–0.48*	+0.61*	–0.03	–0.48*	+0.58*	3.71
	Protein, %	–0.46*	+0.57*	–0.02	–0.41*	+0.56*	–0.01	–0.42*	+0.53*	3.48
	Urea, $mg \cdot L^{-1}$	–93*	–94*	–95*	+109*	+112*	+110*	–3	–1	203
1.2–1.6	Actual milk, kg	+1.8*	–6.1*	–1.4*	+2.9*	–3.4*	+0.9	+3.9*	–4.4*	21.5
	FCM milk, kg	+0.1	–4.8*	–1.5*	+1.3	–1.7*	+0.9	+2.2*	–2.9*	23.2
	Fat, %	–0.75*	+0.75*	–0.01	–0.61*	+0.73*	–0.01	–0.61*	+0.71*	4.59
	Protein, %	–0.61*	+0.58*	–0.01	–0.52*	+0.59*	–0.01	–0.51*	+0.51*	3.42
	Urea, $mg \cdot L^{-1}$	–95	–89	–91	+140	+103	+108	–3	+1	201
>1.6	Actual milk, kg	+1.1*	–4.8*	–6.2*	+1.4*	–6.2*	+2.3*	+2.8*	–2.6*	20.9
	FCM milk, kg	–1.7*	–4.9*	–2.6*	–0.6	–5.7*	+3.9*	+0.5	–1.2*	26.9
	Fat, %	–1.41*	+0.58*	–0.11	–0.85*	+1.19*	+0.27	–1.28*	+0.84*	5.89
	Protein, %	–0.84*	+0.45*	+0.02	–0.63*	+0.64*	+0.01	–0.81*	+0.54*	3.36
	Urea, $mg \cdot L^{-1}$	–95*	–96*	–98*	+115*	+110*	+113*	–6	–5	203

* Difference in the values of particular trait compared to the mean traits for the optimal feeding group significant at $P \leq 0.05$.

>3.6%; >250 250 $mg \cdot L^{-1}$ and 3.2–3.6%) compared to cows fed rationally contained more urea per liter by: 122, 53, and 37 mg, respectively.

Based on the results obtained in the study, it should be stated that the level of protein-energy supply in feed rations for cows was one of the key factors determining the level of milk performance traits.

Variability in milk productivity among cows is a common phenomenon in dairy herds. In Guliński et al. [2018] research on Polish Holstein-Friesian cows, coefficients of variation for milk protein, fat, lactose, and urea levels were 19.5%, 13.8%, 5.3%, and 48.6%, respectively. A number of other scientific studies and official milk performance evaluations also describe similarly high variability in cow milk yield [Sawa et al. 2010, Guliński et al. 2015, Januś and Stanek 2017, Jankowska et al. 2017]. According to Delaby et al. [2009], primiparous cows produce less milk, with lower fat and protein yields compared to multiparous cows. The milk fat content of primiparous cows was significantly lower, but the milk protein content was similar for subsequent lactations.

Commonly, the level of feed ration balancing, lactation stage, and animal production level are considered to be the most important sources of variability in milk yield and its chemical composition in cows [Jenkins and McGuire 2006]. In addition, there are several other factors that influence milk yield in cows, but to a much lesser extent [Guliński 2017]. According to the consensus of many authors, the level of nutrition is a fundamental factor affecting the variability of milk productivity in practical animal breeding [Kowalski and Kamiński 2000, Bilik and Łopuszańska-Rusek 2010, Morales-Almaráz et al. 2011, Barłowska et al. 2012,]. In the experiment of Kvidera et al. [2017], reducing feed intake was accompanied by significant changes in the milk yield of cows. Cows that received 80% and 60% of the demand for nutrients specified in the nutritional standards compared to the control group (100% coverage of the nutritional standard) showed a decrease in daily milk yield by 55% and 33%, respectively. In the milk of these groups of cows, the fat concentration increased by +38% and +13%, respectively, while the protein concentration decreased by –9% and –9%, respectively.

Problems with the rational feeding of cattle can lead to significant variation in milk production efficiency among cows. Results reported by Winnicki et al. [2016] indicate a high deficit of energy in feed rations for cows in the Poland. These authors confirmed energy deficits in feed rations given in as many as 8 out of 10 months of lactation for animals with an average daily milk yield of 49.1 kg. Similarly, the results by Sawa et al. [2010] indicate a low percentage of milk samples indicating bal-

Table 5. Correlation (r) and regression coefficients (b) between milk performance traits of cows fed rations with varying levels of protein-energy balance

Correlated traits	Number of correlated observations, N	Milk performance traits									
		Milk, kg		FCM, kg		Fat, %		Protein, %		Urea, mg · L^{-1}	
		r	b	r	b	r	b	r	b	r	b
Feed rations for which the levels of milk protein and urea indicate a balanced level of energy and protein balance (3.2–3.6% and 150–250 mg · L^{-1}) and:											
– Rations for which the level of milk protein and urea indicate too low protein and varied energy supply:											
<150 and <3.2%	2987	0.06	1.1	–0.01	–0.2	–0.30*	–0.54	–0.58*	–0.59	–0.84*	–94
<150 and >3.6%	2814	–0.41*	–3.6	–0.32*	–2.9	0.39*	0.32	0.79*	0.28	–0.83*	–46
<150 and 3.2–3.6%	7201	–0.09*	–0.6	–0.09*	–0.6	0.01	0.01	–0.05*	–0.01	–0.80*	–31
– Rations for which the level of milk protein and urea indicate too high protein and varied energy supply:											
>250 and <3.2%	2749	0.12*	2.3	0.07	1.2	–0.22*	–0.36	–0.65*	–0.51	0.28*	122
>250 and >3.6%	2874	–0.24*	–2.1	–0.14*	–1.2	0.40*	0.33	0.79*	0.29	0.70*	53
>250 and 3.2–3.6%	6911	0.04	0.3	0.03	0.3	–0.02	–0.02	–0.02	–0.01	0.68*	37
– Rations for which the level of milk protein and urea indicate too low energy and varied protein supply:											
<3.2% and < 150	2987	0.06	1.1	–0.02	–0.2	–0.30*	–0.54	–0.58*	–0.59	–0.84*	–94
<3.2% and >250	2748	0.12*	1.2	0.07	0.6	–0.22*	–0.18	–0.65*	–0.25	0.28*	60
<3.2% and 150–250	10451	0.14*	1.2	0.09*	0.8	–0.18*	–0.14	–0.40*	–0.17	–0.04*	–2
– Rations for which the level of milk protein and urea indicate too high energy and varied protein supply:											
>3.6% and <150	2814	–0.41*	–7.3	–0.34*	–5.8	0.38*	0.65	0.79*	0.58	–0.83*	–92
>3.6% and >250	2874	–0.24*	–1.2	–0.14*	–0.9	0.40*	0.33	0.79*	0.27	0.70*	53
>3.6% and 150–250	11186	–0.24*	–1.7	–0.16*	–1.1	0.29*	0.22	0.59*	0.18	0.01	14

* Correlation coefficient significant at $P \leq 0.01$.

anced energy and protein in feed rations. According to these authors, only 6.6% of milk samples in the second month of lactation showed full energy and protein balance in feed rations. In almost 85% of milk samples from the second month of lactation, a deficiency of energy in the feed ration was indicated. In the study by Januś and Stanek [2017] on Montbeliarde cows, it was shown that 22.9% of milk samples were characterized by full energy and protein balance in the feed ration. Similar to the authors' own research, the highest percentage of samples in this study indicated either energy or protein balance in the feed ration, reaching 35.3% and 68.2%, respectively. Jankowska et al. [2017] demonstrated that the highest percentage of milk samples with the optimum protein and urea content was observed in multiparous cows during 101–200 and 201–300 days of lactation, which had a daily milk yield of 21–30 kg and 31–40 kg in the summer season. According to those authors the increase in daily yield was accompanied by increases (from 2.5% to 37.0%) in the proportion of milk samples being indicative of dietary energy deficiency and (from 0.7% to 36.0%) in the proportion of samples showing energy deficiency and the concurrent protein excess. Guliński et al. [2015] in their research on Polish Holstein-Friesian cows found that 15% of the samples met both criteria for balanced feed rations. In this study, 21% of milk samples contained over 250 mg · L^{-1} of urea and below 3.20% protein. In Januś [2009] research, 43.2% of all analyzed milk samples were characterized by an optimal urea content in milk (141–250 mg · L^{-1}).

According to the results obtained in the study, the authors concluded that the biggest problem in feeding cattle in the evaluated herds was the too low level of energy or too high level of protein in the feed. Salamończyk and Guliński [2015] analyzed the level of urea in milk during the extended lactation period. They found that the highest percentage of milk samples with optimal protein and urea content was found in extended lactation periods (>305 days). The percentage of milk tested during this period, containing urea from 150 to 270 mg · L^{-1} , was 49.26%.

SUMMARY

In summary, the results obtained in this study showed a high and statistically significant influence of the level of energy-protein balance in feed rations on the basic milk performance traits. Evaluating the energy supply of cows, it was found that too low energy levels in rations lead to a decrease in milk chemical composition, while too high levels lead to a decrease in milk yield compared to age-matched cows fed optimally. Milk from cows with energy and protein deficient diets (<3.2% and <150 mg $\cdot$ L^{-1}) contained less fat and protein compared to milk obtained from cows fed optimally, by 0.54% and 0.59%, respectively. Conversely, excess energy feeding (>3.6% and <150 mg $\cdot$ L^{-1}) was accompanied by an increase in fat concentration (+0.66%) and protein (+0.57%) with a decrease in daily milk yield (–7.3 kg). Regarding the level of protein coverage for cows, the study showed that excess protein in the feed rations led to an increase in urea levels in the milk. The level of urea in the milk of cows fed with excess protein in their feed rations (>250 mg $\cdot$ L^{-1} and <3.2%; >250 mg $\cdot$ L^{-1} and >3.6%; >250 mg $\cdot$ L^{-1} and 3.2–3.6%) contained more urea per liter compared to milk from cows fed rationally, with increases of 122, 107, and 109 mg, respectively. The presented results also indicated significant problems with optimally balancing the nutritional needs of cows in the evaluated population. Only 5.2% of the observations indicated complete energy and protein balance. The most commonly occurring rations had an optimal protein level but either too low or too high energy content. The percentage of such identified feeding groups was 24.6% and 27.3%, respectively.

The results obtained in this study are provide a detailed description of the influence of the level of balanced nutritional requirements of cows on their milk production characteristics. On a practical level, these results can be used to optimize the milk production of the basic breed of cattle in Poland, i.e. the Polish Holstein-Friesian. On a theoretical level, they can provide another source of knowledge in the discussion about the possibilities and scale of modifying the chemical composition and milk yield of cows.

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WPŁYW ZRÓŻNICOWANEJ ZAWARTOŚCI BIAŁKA I MOCZNIKA W MLEKU, JAKO BIOMERKERÓW ENERGETYCZNO-BIAŁKOWEGO ZBILANSOWANIA DAWEK POKARMOWYCH, NA POZIOM WYBRANYCH CECH UŻYTKOWOŚCI MLECZNEJ KRÓW RASY POLSKIEJ HOLSZTYŃSKO-FRYZYJSKIEJ

STRESZCZENIE

Celem pracy była ocena wpływu zróżnicowanej zawartości białka i mocznika w mleku na wybrane cechy mleczności krów rasy polskiej holsztyńsko-fryzyjskiej. Badaniami objęto 34 397 zestawów danych dotyczących dziennej produkcji mleka krów rasy polskiej holsztyńsko-fryzyjskiej. Zwierzęta te trzymano w 15 stadach bydła zlokalizowanych na terenie województw mazowieckiego i podlaskiego. W pierwszym etapie badań zwierzęta różnicowano na podstawie poziomu mocznika w mleku oraz procentowej zawartości białka. Zgodnie z przyjętymi założeniami poziom bilansu energetycznego i białkowego dawek pokarmowych określono na podstawie zawartości mocznika i białka w mleku. Ze względu na poziom mocznika wyróżniono trzy grupy krów: <150, 150–250 i >250 mg $\cdot$ L^{-1}. W zależności od zawartości białka w mleku wyodrębniono trzy grupy zwierząt: <3,2%, 3,2–3,6% i >3,6%. Ocenę wpływu zróżnicowanego bilansu energetycznego i białkowego dawek pokarmowych na wybrane cechy mleczności krów przeprowadzono w kolejnych okresach laktacji, poziomie produkcji oraz grupach tłuszczu do białka (F/P). Wyróżniono cztery okresy laktacji, które obejmowały kolejne miesiące laktacji: 1–3, 4–6, 7–10 i 11–18, trzy grupy produkcyjne, określone na podstawie poziomów dobowych wydajności mleka: <20, 20–30 i >30 kg oraz trzy grupy krów o różnym stosunku F/P w mleku: <1,2, 1,2–1,6, >1,6. W kolejnym etapie badań dokonano szczegółowej oceny wpływu zróżnicowanego bilansu energetycznego i białkowego dawek pokarmowych na analizowane cechy produkcyjne mleka. Analizowaną populację krów podzielono na 9 grup w zależności od poziomu zawartości mocznika (mg $\cdot$ L^{-1}) i stężenia białka (%), o różnym stopniu pokrycia zapotrzebowania krów na białko i energię. Za pomocą współczynników korelacji Pearsona i regresji liniowej określono współzależności między cechami użytkowości mlecznej krów żywionych dietami o zbilansowanej zawartości energii i białka oraz ich rówieśników, dla których diety żywieniowe zróżnicowano na podstawie bilansu energetyczno-białkowego. Oceniając zaopatrzenie energetyczne krów w badaniach wykazano, że zbyt niski poziom energii w dietach prowadzi do obniżenia składu chemicznego mleka, natomiast zbyt wysoki prowadzi do spadku wydajności mlecznej w porównaniu z rówieśnikami żywionymi optymalnie zbilansowanymi dietami. Mleko krów niedostatecznie odżywianych energetycznie i białkowo zawierało mniej tłuszczu i białka odpowiednio o 0,54% i 0,59% w porównaniu z mlekiem krów żywionych optymalnie. Nadmiernemu podaży energii towarzyszył natomiast wzrost zawartości tłuszczu (+0,66%) i białka (+0,57%) przy spadku dziennej wydajności mleka (–4,1 kg). Oceniając stopień pokrycia białka przez krowy, badania wykazały, że nadmiar białka w paszy prowadził do wzrostu poziomu mocznika w mleku. Poziom mocznika w mleku krów żywionych nadmiarem białka w paszy (>250 mg $\cdot$ L^{-1} i <3,2%; >250 mg $\cdot$ L^{-1} i >3,6%; >250 mg $\cdot$ L^{-1} i 3,2–3,6%) zawierał więcej mocznika na litr w porównaniu z mlekiem od krów karmionych zbilansowaną dietą, przy wzroście odpowiednio o 127, 107 i 109 mg.

Słowa kluczowe: krowy mleczne, dawka pokarmowa, bilans, cechy mleczne

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