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The effect of daily milk production on the milk composition and energy management indicators in Holstein–Friesian and Simmental cows

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Abstract: The present study investigated the effect of daily milk production on the content of crucial milk components, body condition, and the level of energy management parameters in the blood and milk in Holstein–Friesian (HF) and Simmental (SIM) cows. Within each breed, the animals were divided into two groups: the HF-L group was HF cows with daily milk production of ≤31 kg and the HF-H group was those with daily milk production of >31 kg. The SIM-L was SIM cows with daily milk production of ≤27 kg and the SIM-H group was those with daily milk production of >27 kg. The intensity of reserve fat accumulation was assessed by using body condition scoring. Samples of milk were taken every 30 days of lactation and the content of total protein, casein and κ-casein, fat, lactose, dry matter, and urea were determined. Blood samples were taken on days 10, 40, 70, 100 and 130 of lactation to determine the content of glucose and beta-hydroxybutyrate. The obtained results suggest that, in spite of the lower milk production, the Simmental cows had the more favorable energetic homeostasis of the organism. The values of casein and κ-casein indicate better suitability of the raw material obtained from Simmental cows for cheese processing. From a practical point of view, higher Holstein–Friesian cows' average daily yield and a relatively small difference in the content of the total protein compared to Simmental cows' milk suggest its use primarily as consumption milk.

Key words: Milk production, milk composition, Holstein–Friesian, Simmental, body condition, energy management, correlation

1. Introduction

According to the current data, the population of dairy cattle in milk recording in Poland is over 753 000 heads (1). The invariably predominant dairy breed is the Holstein–Friesian (over 660 000 of the recorded cows), but there is still a growing trend to breed a colored breed such as Simmental (over 10 700 of recorded cows). These breeds settle for feed with lower nutritional value, which is essential for regions where, due to climatic conditions, the production of highly nutrient concentrated feed is difficult, as well as for farms preferring sustainable milk production systems. As is well known, environmental conditions should be recognized as essential factors influencing the emergence of the genetic potential in dairy cattle (2,3). Over the years, particular attention was paid to the intensive selection of the Holstein–Friesian breed. The primary focus was on increasing the intensity of milk secretion and improving body conformation (4,5). Unfortunately, this process was not accompanied by a sufficiently rapid increase in the intensity of metabolic changes in the rumen or the mechanisms that allow for the use of energy stored in the body in the form of adipose tissue. This increased the risk of disturbances in the mechanisms regulating homeostasis of the body's energy; therefore, nowadays, the most common disorders in cows have a metabolic etiology. They manifested, but were not limited to, excessive mobilization and catabolism of the adipose tissue (6) and higher glucose demand (7). Metabolic complications lead to a disturbance in the function of the organism on many levels. This not only deteriorated milk secretion and lowered lactation persistency but also caused excessive immunosuppression, which is induced under such circumstances, increasing, among others, the susceptibility to mastitis and decreasing systemic immunity (8,9). Moreover, the synthesis of casein, which occurs in milk-producing cells, can be destabilized, which negatively affects dietary values and milk processing suitability. Metabolic disorders of the rumen and energy homeostasis in the body of cows have an impact on the efficiency of milk protein production, and this may be important for the transfer of nitrogen into the environment (10).

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The present experiment tested the hypothesis regarding the effect of daily milk production on the content of crucial milk components, body condition, and level of energy management parameters in the blood and milk in Holstein–Friesian and Simmental cows.

2. Materials and methods

The present study was conducted under a research protocol approved by the III Local Ethical Committee in Warsaw, Poland, No. 70/2013 from 19 December 2013. The experimental animals were obtained from two farms (I and II) with similar technical infrastructures, housing, and feeding conditions. The farms were located in eastern Mazovia. The structure of the herds was as follows: farm I, 120 Holstein–Friesian (HF) cows (75% during lactation); farm II, 15 HF cows and 29 Simmental (SIM) cows (75% during lactation). In both farms the cows were kept in free-standing barns. The silage feed was administered on the feeding passage and was regularly pushed back to the cattle. The cows were milked twice every 24 h in milking parlor. In the experiment, 58 HF cows (2.9 ± 0.9 lactations) from farm I and 23 SIM cows (3.4 ± 0.9 lactations) from farm II were used. Within each breed, the animals were divided into two groups that differed in terms of daily milk production. The daily milk production limit (kg/day) was median calculated for this indicator in each breed group; for HF it was set at 31.0 kg/day, and for SIM at 27.0 kg/day. The HF-L group was HF cows with daily milk production of ≤31 kg and the HF-H group was those with daily milk production of >31 kg. In turn, the SIM-L group was SIM cows with daily milk production of ≤27 kg and the SIM-H group was those with daily milk production of >27 kg. The intensity of reserve fat accumulation was assessed at the beginning (1–3 days after calving) and on day 30 of lactation by using body condition scoring (BCS) in the range from 1 to 5 points (1 point - emaciated cow, 5 points - fattened cow). The BCS was assessed by three people. The average body weight of the cows from farm I was 658 ± 24.2 kg and from farm II it was 598 ± 22.5 kg. The diets were formulated on the basis of the maintenance requirement of animals specified by the French National Institute for Agricultural Research (INRA) feeding standards with the use of INRAtion Software for Ruminant Diet Calculation, version 2.03 (DJ Group, Krakow, Poland) based on the earlier chemical analysis of feeds and calculation of their nutritional value. All cows received a complete feed mix, the base of which (55%) was silage (maize, alfalfa, haysilage), concentrated feed containing soybean and rapeseed middlings (45%), straw shred (0.3 kg/cow per day) and mineral–vitamin formulations. The average energy density of the dry matter of the food dose was 6.87 ± 0.29 MJ net energy for lactation (NEL). Dry matter intake (DMI) was predicted by the equation: $DMI (kg/day) = (0.372 \times FCM + 0.0968 \times BW^{0.75}) \times (1 - e^{(-0.192 \times (WOL + 3.67))})$. For the first 100 days of lactation, the diet was enriched with buffering additives. The nutritional value of the feed was determined each time after the silos with silage were opened. Throughout the year, the cows were fed according to the TMR system. The diet was set for cows weighing about 650 kg for HF and 600 kg for SIM and taking into consideration the coverage of the energy requirement for the average amount of milk secreted during the experiment period. The information on the amount of milk produced and the fat contained was determined on the basis of previous lactations. Samples of raw milk were taken in sterile bottles from day 7 after calving to about 6 months of lactation (every 30 days on average). Milk samples complying with the EU standards (11) were included in the study. The milk analyzer Bentley Combi 150 (Bentley Instruments Inc., Chaska, MN, USA) was used to determine the basic components of milk: the total protein, fat, lactose, dry matter, and urea. The content of the total casein was measured after precipitation and purification of casein in acetate buffer (pH 4.6, 20 °C). In the resulting sediment (casein), the content of the total nitrogen was determined using the Kjeldahl method (12), and converted to the total protein content using the equivalent of 6.25. The determination of κ-casein was performed using an HPLC-VARIAN liquid chromatograph (Varian Inc., Palo Alto, CA, USA). The resulting isobat was dissolved in TRIS-HCl buffer (pH 8.0). Protein separation was carried out on an Aeris XB-C-18 column, 3.6 µm, 250 × 4.6 mm (Phenomenex Inc., Torrance, CA, USA) at the temperature of 40 °C (flow: 1.5 mL/min, A: 0.1% trifluoroacetic acid aqueous solution (TFA), B: 0.1% TFA solution in acetonitrile). The concentration of κ-casein was determined on the basis of a calibration curve drawn using the bovine κ-casein standard (Sigma-Aldrich, St. Louis, MO, USA).

Blood samples were taken before the morning feeding, from the abdominal vein, on days 10, 40, 70, 100, and 130 of lactation. The content of glucose in the blood was determined using the original Randox kits (Randox Laboratories Ltd, Crumlin, UK) and a UV-Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). To determine beta-hydroxybutyrate (BHBA), the samples were centrifuged at 1500 × *g* at 4 °C for 20 min. The supernatant was collected and stored at –20 °C until it was analyzed for BHBA using original Randox kits and the UV-Vis spectrophotometer.

The data were statistically analyzed using Statistica 12.5 by analysis of variance. All the results given in tables as weighted mean with the standard error of the mean (SE) were subjected to two-factor analysis of variance (breed - B × production group - GP) to evaluate tested parameters (P value). Differences between means were studied using

Tukey's method (the level of significance was set at 5%). Pearson's correlation coefficients (r) were calculated between the selected features ($P < 0.05$) within the breed (R).

3. Results

Table 1 summarizes the results of the cows' condition (BCS) and the content of some milk components in the study breed groups based on the mean daily milk production. The data indicate that in both breeds the strong body condition of cows at the beginning and after 30 days of lactation was a significant factor related to the amount of milk secreted (HF-H and SIM-H groups). It should also be noted that the SIM breed showed greater tendency to keep fit compared to the HF cows from both production groups. The release of energy from reserved fat in the first 30 days of lactation was milder in the SIM cows. This was proved by small differences, which in the SIM-L and SIM-H groups were 0.12 and 0.19 BCS points, respectively, and in the HF cows (HF-L and HF-H) 0.21 and 0.24 BCS points. However, the differences in daily milk production between groups within each breed were similar (11.7 kg on average). The trend in the BCS suggested a stronger correlation with daily milk production in the HF cows. This was confirmed by correlation coefficient values (Table 2). The results for relative change in the BCS (Table 1) confirmed larger decrease in the BCS value in the HF cows compared with that in the SIM cows.

The results of milk composition shown in Table 1 indicate that in both breeds the concentration of the total protein and casein decreased with the increase in milk production. However, the analysis of the results indicated that the proportion of these components in the milk from the SIM-H cows decreased to a lesser extent. As for the total protein, the difference was 0.05% and the content of casein decreased by 1.33%. This was a more favorable result

Table 1. Energy balance, body condition, and milk composition in the HF and SIM cows.

Parameter	Breed (B) / Production group (GP)				SE	B × GP P <
	HF		SIM			
	HF-L	HF-H	SIM-L	SIM-H		
Number of individuals	30	28	11	12		
Daily milk production (kg)	25.9^{a}	37.8^{b}	21.3^{c}	32.8^{d}	0.36	0.000
Energy (MJ NEL):						
Request	114.9^{a}	144.3^{b}	103.0^{c}	130.5^{d}	0.29	0.019
Intake	100.8^{a}	97.6^{b}	107.4^{a}	141.0^{c}	0.11	0.008
Balance	-14.1^{a}	-46.7^{b}	-4.4^{c}	-10.5^{a}	0.09	0.023
DMI (kg/day)	16.3^{a}	15.1^{b}	17.8^{c}	16.6^{a}	0.04	0.039
BCS – Day 1 of lactation (point)	2.54^{a1}	2.85^{b1}	2.51^{a}	2.90^{c1}	0.02	0.004
BCS – Day 30 of lactation (point)	2.33^{a2}	2.61^{b2}	2.39^{a}	2.71^{c2}	0.04	0.009
Relative change in BCS (%)	8.27^{a}	8.42^{a}	4.78^{b}	6.55^{c}	0.08	0.021
Number of milk samples	150	140	55	60		
Milk components:						
Total protein (%)	3.34^{a}	3.27^{b}	3.35^{a}	3.30^{b}	0.02	0.015
casein (% of total protein)	75.48^{ac}	73.94^{b}	76.21^{c}	74.88^{a}	0.31	0.028
κ-casein (% of total protein)	9.64^{a}	9.38^{b}	9.97^{c}	9.78^{c}	0.20	0.031
Fat (%)	3.76^{a}	3.56^{b}	4.01^{c}	3.90^{c}	0.05	0.016
Lactose (%)	4.72^{a}	4.75^{a}	4.59^{b}	4.68^{c}	0.02	0.007
DM (%)	12.08^{a}	12.04^{a}	12.26^{b}	12.13^{c}	0.03	0.023

SE- standard error of the mean; DMI- dry matter intake; BCS- body condition scoring; HF- Holstein–Friesian cows, SIM- Simmental cows; HF-L- daily milk production ≤31 kg, HF-H- daily milk production >31 kg, SIM-L- daily milk production ≤27 kg, SIM-H- daily milk production >27 kg; ^{a,b,c,d}- means in the row with different letters differ significantly ($P < 0.05$); ^{1,2}- means in the column with different superscripts differ significantly ($P < 0.05$)

Table 2. Pearson's correlation coefficients for daily milk production, BCS, the total protein and BHBA with energy management parameters.

	HF	SIM	HF	SIM	HF	SIM	HF	SIM
Parameter	Daily production (kg)		BCS (points)		Total protein (%)		BHBA (mmol L^{-1})	
Daily production (kg)	-	-	0.903*	0.494*	–0.156	–0.144	-	-
Blood parameters:								
Glucose (mmol L^{-1})	–0.915*	–0.565*	–0.751*	–0.723*	0.136	-	–0.369*	–0.607*
BHBA (mmol L^{-1})	0.401*	0.854*	0.342*	0.307*	–0.187	-	-	-
Milk parameters:								
Urea (mmol L^{-1})	0.238*	0.125	0.205	-	-	-	0.264*	-
Fat/protein	0.308*	-	0.281*	-	–0.259*	–0.320*	0.547*	0.139

HF- Holstein–Friesian cows, SIM- Simmental cows; BCS- body condition scoring; BHBA- beta-hydroxybutyrate; *- $P < 0.05$

compared to the Holstein–Friesian milk (these values were 0.07% and 1.54%, respectively). The data (Table 1) showed that an increase in the mean amount of milk excreted per day leads to a decrease in the content of this ingredient in both breeds; the differences in the content of κ-casein between the HF groups were statistically significant. The lack of a strong correlation between daily milk production and the total protein content (Table 2) can be explained by the fact that in the first weeks of lactation the demand is covered from the fat and protein accumulated in the body. The analysis of the fat and the lactose (Table 1) showed higher content of fat and the similar content of lactose in milk secreted by the HF-L cows compared with that of the HF-H cows. The milk secreted by the SIM cows with higher daily production (SIM-H) had on average 0.11% lower fat content and 0.09% higher lactose content. These results were related to daily milk production and glucose content interrelation (Table 2). Significant correlations of these parameters were identified in both study breeds ($P < 0.05$) but the size of the correlation for the HF cows was definitely stronger. The level of glucose and BHBA concentration depending on the size of production in the study breeds is summarized in Table 3. Both SIM groups (SIM-L and SIM-H) had significantly higher concentration of this parameter compared to that of the HF breed. In turn, the analysis of BHBA data showed its greatest concentration in the HF-H group. The BHBA concentration was correlated ($P < 0.05$) with the BCS parameter in both breeds of cow (Table 2), and the intensity of the correlation was similar. In turn, the daily milk production for both the HF and the SIM cows was significantly correlated with BHBA but the coefficient value for this interrelation in the SIM breed was much higher.

The mean values of fat to protein ratio in the milk of the studied cows (Table 3) did not show any carbohydrate abnormalities in the rumen or digestive system disorders. The values were within typical limits (1.1–1.3). The results of urea (Table 3) showed that the HF-derived milk had higher content of this ingredient. The differences also indicated the higher content of milk urea in individuals of both breeds producing more milk (HF-H and SIM-H) by 0.137 and 0.165 mmol L^{-1} in the SIM and HF cows, respectively.

4. Discussion

From the point of view of the profitability of production, the important factors are quantity and quality of milk. The average amount of milk excreted per day is rather typical of the genetic potential of a given breed of dairy cow. As it can be deduced based on the BCS, to a large extent, it should also be linked to the efficiency of managing the energy stored in fat. The trend in BCS observed in the present experiment suggests stronger correlation with daily milk production in HF cows. The results of our research correspond to those reported by Lawrence et al. (13). Interesting is the fact that in both groups of HF cows (HF-L and HF-H) the values of relative change in BCS were very similar (Table 1), while there were significant differences between the production groups of the SIM breed. The results obtained may indicate that in the HF cows the mechanisms starting the body energy transformations were more sensitive to changes in daily milk production.

Casein is an important ingredient of the total protein in milk. Fleischer et al. (14) and Ingvartsen et al. (15) demonstrated that an increase in the casein content in milk should be achieved primarily by improving its

Table 3. Energy management parameters in the blood and milk of the HF and SIM cows.

Parameter	Breed (B) / Production group (GP)				SE	B × GP P <
	HF		SIM			
	HF-L	HF-H	SIM-L	SIM-H		
Number of blood samples	30	28	11	12		
Blood parameters:						
Glucose (mmol L^{-1})	2.865[a]	2.721[b]	3.665[c]	3.176[d]	0.034	0.010
BHBA (mmol L^{-1})	0.867[a]	1.435[b]	0.611[c]	0.765[d]	0.020	0.001
Number of milk samples	150	140	55	60		
Milk parameters:						
Urea (mmol L^{-1})	4.208[a]	4.373[b]	3.801[c]	3.938[d]	0.024	0.034
Fat/protein	1.19[a]	1.08[b]	1.15[c]	1.20[a]	0.03	0.026

SE- standard error of the mean; HF- Holstein–Friesian cows, SIM- Simmental cows; HF-L- daily milk production ≤31 kg, HF-H- daily milk production >31 kg, SIM-L- daily milk production ≤27 kg, SIM-H- daily milk production >27 kg; BHBA- beta-hydroxybutyrate; [a,b,c,d]- means in the row with different superscripts differ significantly ($P < 0.05$)

proportion to the overall protein, but with relatively balanced production and constant lactation. This method of milk production improvement is of greatest importance to the health of cows. In addition, according to Hallen et al. (16), it is crucial to increase the content of casein (the efficiency of its synthesis) in milk intended for cheese making. In this respect, it is particularly important to have a greater proportion of κ-casein (κ-CN), the content of which correlates with the higher cheese yield, stronger curd, and lower outflow of casein fraction with whey. Our results are partially consistent with those obtained by Heck et al. (2) and Bobe et al. (17). In these studies, the authors show that genotype has a higher impact than milk yield on κ-CN concentration and the share of casein in the total protein. It should be borne in mind, however, that due to some synergy the amount of the produced milk may be an additional factor reducing the content of its key ingredients. Thus, in some breeds, the smaller amount of milk secreted per day does not always have to decrease the economic efficiency because a greater content of the casein fraction gives a more favorable economic effect resulting from higher curd productivity (2).

The pattern of lactation, especially to reach the production peak, is an important aspect influencing the homeostasis of the body. The adaptability of cows connected with the resilience to energy deficit in the period of increased milk secretion can be only partially compensated. However, a negative energy balance always results in the intensive activation of body fat reserves. Glucose, which is a very important element of energy management, takes part, among others, in the synthesis of lactose (7). Its concentration in the blood is a direct indicator of energy changes but, as shown by Roche et al. (9), the lowering of its concentration may be a consequence of systemic immunity disorders (production stress, inflammation). Therefore, the intensity of processes during lactation can be analyzed on the basis of fat, lactose, glucose, and BHBA contents. The analysis of the BHBA in the present study showed the highest content of this component in milk secreted by the HF-H cows and this trend may be explained by the increased mobilization of body energy reserves (the largest decrease in BCS) in the HF-H cows. Trends in glucose concentration in the present experiment are consistent with those demonstrated by Bauman and Currie (7) and Kappel et al. (18), who found that the increased demand for glucose in the studied animals resulted from an intensive synthesis of milk sugar. Some recent studies have shown that in cows the coverage of enormous demand for glucose after the initiation of lactation can be met through the mechanisms of hepatic glucose synthesis (19). In light of this, differences in the glucose levels may suggest that the SIM cows had greater ability to adapt to the increased milk secretion. The higher glucose and lower BHBA levels may indicate higher efficiency of the liver in these cows. On the other hand, Galvao et al. (20) have found that increased glucose demand can be induced by excessive immunosuppression, which in high-performance individuals is activated by progressive weakening of the body. In the context of the above studies, our results may suggest that lower blood glucose levels were due to the synergistic effect of lactose synthesis and the amount of milk excreted. It should be noted, however, that in the SIM cows the interaction of these factors was less intense. Unlike our research, Weber et al. (19) found lower blood glucose concentration in cows with an average daily yield of 41.45 kg.

Metabolic changes in the rumen and liver are the result of protein–energy balance and the intensity of milk secretion. Therefore, the first weeks of lactation are a period in which cows are at the greatest risk of homeostasis destabilization. The highest concentration of BHBA in the HF-H cows in the present experiment may indicate the most intense release of energy stored in fat in this group of animals. In the case of BHBA, the limit value assumed as typical of ketosis (up to 1400 mmol L^{-1}) was exceeded. In this respect, the results correspond to those obtained by Fleischer et al. (14) and Ingvartsen et al. (15), who showed, among other things, the strong correlation between milk yield and metabolic diseases, especially in the early lactation period. Regarding the content of key milk components, this fact plays an important role as a strong link has been confirmed between the function of the rumen and its bacterial flora, and the formation of major milk protein precursors and pH (21–23). The studies conducted by Prendiville et al. (24) and Mapekula et al. (25) demonstrated that the amount of produced milk and the associated content of main components were most often connected with higher intake of dry matter. This prolongs the predigestion process, which in turn has an influence on the bacterial flora and the intensity of ruminal changes (3). According to Sun and Gibbs (26), this can decrease the activity of stearyl-CoA desaturase (the regulator of lipid metabolism) and affect the amount and quality of milk fat. The possible slowdown of food passage may additionally lead to lower blood glucose concentration in the HF-H and SIM-H cows, which was confirmed in the studies. This also has implications for protein milk synthesis because the stimulation of the blood flow in the mammary gland causes glucose retention, which probably has an impact on the efficiency of milk protein synthesis (27).

Ammonia is one of the more harmful constituents of the ineffective bacterial protein breakdown. Based on the results of the content of milk urea, which is synthesized from ammonia and carbon dioxide in the liver, it may be concluded that in the rumen of the HF breed, the efficiency of the development of substrates by bacteria was lower. Weber et al. (19) found that changes in the content of milk urea in cows depend on the lactation phase rather than on the amount of milk secreted per day. The studies conducted by Weber et al. (19) indicate that the content of urea was from 263 mg/kg on days 28 and 56 of lactation to 210 mg/kg on day 90. At the same time, in analogous lactating periods, the level of daily production was similar, from 41.45 kg/day (on days 28 and 90 of lactation) to 40.6 kg/day (on day 56 of lactation).

The results obtained in the present experiment suggest that, in spite of the lower milk production, the Simmental cows had the more favorable energetic homeostasis of the body. The outcomes may significantly expand our knowledge of the dairy industry and thus improve the raw material obtained from dairy cows. From a practical point of view, higher Holstein–Friesian cows' average daily yield and a relatively small difference in the content of the total protein compared to Simmental cows' milk suggest its use primarily as consumption milk. On the other hand, the values of casein and κ-casein indicate a better suitability of the raw material obtained from Simmental cows for cheese processing.

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