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Studies upon genetic and environmental factors affecting the cholesterol content of cow milk. II. Effect of silage type offered

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The aim of the study was to determine the effect of the type of silage (wilted grass *vs*. whole maize plants) offered to high-yielding dairy cows on cholesterol content of their milk. Silage type did not affect the cholesterol level as expressed either in mg/100 ml milk or as mg/g milk fat. However, the significant relationships were identified between the cholesterol content of milk and stage of lactation, milk somatic cell count, daily milk yield, fat content of milk and the amount of fat yielded daily.

KEY WORDS: cholesterol /dairy cows/ milk / silage type

The cholesterol content of bovine milk is determined both by genetic (breed and milk yield) and environmental factors [Verma and Prasad 2000, Tomaszewski 2005]. The results of the few studies on this problem published so far indicate also a significant relation between the cholesterol content of milk and the age of cows [Tomaszewski and Hibner, 2001], stage of lactation [Turk *et al*. 2003], cows' physiological status [Polat and Cetin 2001] and season of the year [Calamari *et al.* 1999, Paura *et al.* 2003]. Most authors report the effect of season of the year on the cholesterol content of milk without paying attention to the fact that it is related to the feeding regimen. This is confirmed by Tomaszewski [2005], who showed that the effect of season on

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the cholesterol content of milk was not significant if the cows were fed uniformly over the whole year.

In the literature available there are no reports from studies conducted on high yielding dairy cows and analysing the effect on the cholesterol content in milk of diets containing different forages. As silages made of whole maize plants or from wilted grass are the basic feeds used in modern feeding systems for high-yielding dairy cows, the present study aimed at determining the effect of diets containing such feeds on the yield and chemical composition of milk, with special reference to the cholesterol content.

Material and methods

The investigations were conducted over a period of 200 days of winter nutrition on 40 Polish Holstein-Friesian (Black-and-White) cows-in-milk with a share of Holstein-Friesian genes exceeding 87.5%. Twenty cows (group 1) were offered a diet in which, as the alone forage, the wilted grass silage (33% dry matter) was introduced. The silage dry matter daily intake by group 1 amounted to about 10 kg per cow (equivalent of 8.0 JPM and 720 g BTJ). Another 20 cows (group 2) were offered a diet in which the only forage was the whole plant maize silage (35% dry matter). The silage dry matter daily intake by group 2 amounted to about 9 kg per cow (equivalent of 8,1 JPM and 590 g BTJ). Both silage-containing diets were balanced with the same type of concentrate and mineral-and-vitamin additive to meet the cows' requirements according to the INRA [2001] feeding standards. The nutritive value of the feeds offered was determined on the basis of proximate analyses performed at the Institute's laboratory, using the INWAR D.J. Group software. The cows were kept tied on standings adapted for individual feeding, with no bedding. The feed was offered twice a day, every 12 hours, and water was available continuously in automatic waterers. Between feedings the animals spent several hours on a paddock. The cows were machine-milked twice daily, at 12 hour intervals. Milk samples for analyses were obtained once a month, during test milkings. From each cow a collective daily sample was prepared, mixing milk from the morning and evening milkings proportionally to the yield obtained. The samples were analysed for the basic chemical composition, somatic cell count (SCC) and total cholesterol content. On the basis of the of milk yielded and its chemical composition a calculation was made of the amount of individual components secreted in milk during 24 hours.

The saponification of fat and extraction of cholesterol from milk was performed according to Fletouris *et al.* [1998] and quantitative determination of cholesterol content was conducted using the colorimetric method by Searcy and Berquist [1960].

The fat, protein and lactose contents of milk were determined using a MilkoScan 104A/B, while the SCC with a Fossomatic 90 device.

Statistical evaluation of results was done using the GLM and corr procedure of SAS Version 9.1.3 for Windows [SAS, SAS/STAT 2002-2003]. The base model included stage of lactation, lactation parity, somatic cell count (SCC) and diet type as fixed effects. A fixed regression on milk yield was used. Moreover, to calculate the total cholesterol in daily milk yield, cholesterol as mg/100 ml milk and cholesterol as mg/g milk fat fixed regressions on fat content and daily fat yield were used.

Three lactation stages were considered: 1 – 6-60 days, 2 – 61-210 days and 3 – above day 210, and three lactation parities with parity III including lactation III and later. To determine the effect of SCC, four classes of milk were distinguished: 1 – with less than 100, 2 – with 101-400, 3 – with 401-800 and 4 – with more than 800 thousand cells/ml. The SCC values were transformed into the natural logarithm scale (lnSCC). To determine the effect of diet two classes were established: 1 – containing wilted grass silage, and 2 – containing whole maize silage.

Results and discussion

The cholesterol content of milk, expressed both as mg/100 ml milk and mg/g milk fat was found to be significantly related to the stage of lactation, SCC, daily milk yield, fat content of milk and daily yield of milk fat (Tab. 1).

Table 1. The effect of factors analysed on the cholesterol content of milk

Factor analysed	Cholesterol		
	mg/100 ml milk	mg/day/cow	mg/g fat
Lactation parity	ns	ns	ns
Lactation stage	x	xxx	xx
Silage type	ns	ns	ns
SCC/ml	xxx	xx	xxx
Daily milk yield (kg)	x	xxx	x
Fat content of milk (%)	x	ns	xx
Daily milk fat yield (g)	x	ns	x

x – P≤0.05; xx – P≤0.01; xxx – P≤ 0.001; ns – non significant.

The type of diet offered, differing as regards forage offered (wilted grass silage *vs*. maize silage) affected neither the cholesterol content of milk expressed as mg/100 ml milk and mg/g milk fat, nor the amount of cholesterol in the daily milk yield (Tab. 2). In the literature available there were found no results of studies conducted on the basis of a similar experimental design. The earlier investigations based on different designs indicate that the composition of diets offered to cows affects the composition of lipids, including the cholesterol concentration [Fahey *et al*. 2002, Reklewska *et al*. 2002, Nałęcz-Tarwacka *et al*. 2008]. A relation was also demonstrated between the type of feed offered and cholesterol content of blood serum of cows [Cole *et al*. 2001, Pieszka and Brzóska 2001, El-Hafez *et al*. 2002]. However, as a relation between the cholesterol content of the blood serum and milk has not yet been documented, there is

Table 2. Least squares means (LSM) and standard errors (SE) for three measurements of cholesterol content across factors analysed

Factor analysed	No. of samples	Cholesterol					
		mg/100 ml milk		mg/day/cow		mg/g fat	
		LSM	SE	LSM	SE	LSM	SE
Group							
Feeding grass silage	195	20.57	0.63	3608	76	5.03	0.10
Feeding maize silage	222	21.10	0.44	3614	75	5.01	0.10
Lactation parity							
I	61	20.57	0.39	3671	122	5.05	0.16
II	118	21.11	0.47	3633	86	5.09	0.11
III	238	20.58	0.48	3529	65	4.92	0.09
Lactation stage							
1	48	19.26^{A}	0.76	3255^{A}	148	4.58^{A}	0.19
2	192	21.19^{B}	0.36	3769^{B}	7	5.18^{B}	0.09
3	177	21.70^{B}	0.42	3808^{B}	081	5.30^{B}	0.11
SCC/ml/milk							
class 1	97	19.70^{A}	0.50	3419^{A}	97	4.76^{A}	0.13
class 2	139	20.61^{A}	0.45	3623^{A}	87	5.02^{A}	0.11
class 3	55	20.41^{A}	0.62	3574^{A}	120	4.94^{A}	0.16
class 4	126	22.15^{B}	0.44	3828^{B}	86	5.36^{B}	0.11

^{AB}Within factors and traits. LSMs bearing different superscripts differ significantly at $P\leq0.01$.

no justification to assume, that a change in the cholesterol concentration of blood leads to changes in its concentration of milk. The differences occurring in the cholesterol concentration of milk are determined by the concentration and type of nutrients of the feed offered. Among much else, it was demonstrated that soybean protein leads to a decrease, while the addition of casein proteins to an increase in cholesterol concentration of milk [Hanczakowski 1998]. The results of studies conducted both on humans and on monogastric and ruminant animals, indicate that the cholesterol level of blood depends not only on the amount of fibre consumed, but also on its quality [Hicks *et al*. 1995, Gerhardt and Gallo 1998, Singh *et al.* 2002]. Tomaszewski [2005] reported a similar relation between the amount of fibre consumed by cows and the cholesterol content of milk: a decrease in the crude fibre intake from 287 to 190 g per kg of milk produced led to an increase in the cholesterol content per g of milk fat by about 13%. Nałęcz-Tarwacka [2006] demonstrated that the cholesterol content of milk in cows fed processed feeds over the whole year according to the TMR system, was significantly higher when compared with other diets, containing pasture grass during the summer season. The results of the present study, showing no differences in the cholesterol content of milk between cows fed diets containing silages from wilted grass or whole maize plants are favourable, as both those forages are the principal components of modern systems of feeding cows.

However, the diets offered did affect significantly the milk yield and dry matter content of milk, the latter determined by the higher level of protein and lactose; the diet containing maize silage had a favourable effect on the level of both components mentioned (Tab. 3). The cows fed the diet based on whole plant maize silage (group 2) consumed more net energy for milk production than those fed the grass silage (group 1). Simultaneously it was confirmed that it is difficult in practice to make a highly precise evaluation of the energy content of feeds composing the animals' diet, using computer software to determine the nutritive value of feeds on the basis of results from chemical analyses.

Pearson's coefficients of simple correlation showed that the content of cholesterol, expressed as mg/100 ml of milk was negatively correlated with the daily milk yield and only slightly related to milk dry matter, fat and protein (Tab. 4). The amount of cholesterol expressed in mg/g of milk fat was significantly negatively correlated with the content of dry matter, fat and protein, but did not with the daily milk yield. The amount of cholesterol secreted in milk during 24 hours was to the greatest degree correlated with the daily milk yield and negatively with the dry matter, fat and protein content of milk.

The cholesterol content of milk, expressed both as mg/100 ml milk and mg/g milk fat, was significantly related to the stage of lactation (and thus also to the amount of milk yielded per day), fat content (%) and fat yield of milk over 24 hours (g) as well as to the SCC (Tab. 1). With the progress of lactation the cholesterol content of milk increased by about 13% (Tab. 2). Similar

Table 3. Least squares means (LSM) and standard errors (SE) for daily milk yield and milk component contents across factors analysed

Factor analysed	No. of samples	Daily milk yield (kg)		Component (%)							
				total solids		fat		total protein		lactose	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Feeding grass silage	195	18.3^{A}	0.4	12.13^{A}	0.08	4.14	0.05	3.29^{A}	0.03	4.70^{A}	0.03
Feeding maize silage	222	20.9^{B}	0.3	12.46^{B}	0.07	4.16	0.05	3.49^{B}	0.03	4.81^{B}	0.03
Lactation parity											
I	61	19.2	0.6	12.27^{AB}	0.12	4.04^{A}	0.08	3.38^{AB}	0.05	4.85^{A}	0.04
II	118	19.8	0.4	12.12^{A}	0.09	4.11^{A}	0.06	3.33^{A}	0.03	4.68^{B}	0.03
III	238	19.9	0.3	12.49^{B}	0.07	4.29^{B}	0.04	3.45^{B}	0.02	4.74^{C}	0.02
Lactation stage											
1	48	25.7^{A}	0.6	12.32^{AB}	0.15	4.18^{A}	0.10	3.35^{A}	0.06	4.80^{A}	0.05
2	192	19.6^{B}	0.3	12.14^{A}	0.07	4.02^{B}	0.05	3.35^{A}	0.03	4.77^{AB}	0.02
3	177	13.6^{C}	0.3	12.42^{B}	0.08	4.24^{A}	0.06	3.47^{B}	0.03	4.71^{B}	0.03
SCC/ml milk											
class 1	97	21.9^{A}	0.4	12.38	0.10	4.18	0.07	3.37	0.04	4.83^{A}	0.03
class 2	139	19.2^{B}	0.4	12.26	0.09	4.12	0.06	3.38	0.03	4.76^{A}	0.03
class 3	55	18.8^{B}	0.6	12.37	0.12	4.17	0.08	3.40	0.04	4.80^{A}	0.04
class 4	126	18.7^{B}	0.4	12.17	0.09	4.12	0.06	3.41	0.03	4.64^{B}	0.03

^{AB}Within factors and traits. LSMs bearing different superscripts differ significantly at $P \leq 0.01$.

Table 4. Pearson's correlation coefficients between three measurements of cholesterol content of milk and milk yield and chemical composition

Item	Milk yield (kg)	Total solids (%)	Solids non fat (%)	Fat (%)	Total protein (%)
Cholesterol mg/100 ml	-0.24[xxx]	0.03	-0.06	0.10[x]	0.14[xx]
Cholesterol mg/day	0.78[xxx]	-0.41[xxx]	-0.36[xxx]	-0.33[xxx]	-0.53[xxx]
Cholesterol/fat (mg/g)	0.03	-0.50[xxx]	-0.32[xxx]	-0.51[xxx]	-0.23[xxx]

[x]$P \leq 0.05$; [xx]$P \leq 0.01$; [xxx]$P \leq 0.001$.

trend in the content of cholesterol was observed in relation to the SCC. An increase in the cholesterol content of milk with the progress of lactation was observed also by Son *et al.* [1996], Tomaszewski and Hibner [2001] and Turk *et al*. [2003] while with the increase in per cent of HF genes in the genotype – by Tomaszewski [2005]. An increase in the cholesterol concentration of the milk in cows (by about 19%) with the increasing share of HF genes was observed also in part I of the present report [Strzałkowska *et al*. 2009] in which the cows were characterized by a lower share of HF genes (about 50%) and lower milk yield (about 3800 kg/lactation) compared to those used in the current part II in which the share of HF genes exceeded 87% and mean milk yield was almost two times higher (about 6050 kg/lactation).

In the present study no effect was identified of the cows' age (lactation parity) on the cholesterol content of milk (Tab. 2), while Tomaszewski and Hibner [2001] and Tomaszewski [2005] reported such a significant relation to occur. Tomaszewski [2005] indicated that the cholesterol content of milk of cows decreased with the animals' age, the difference between cows in their 3rd *vs*. 9th lactation amounting to about 7%. A more detailed description of the effect of non-nutritive factors, *i.e*. stage of lactation and season, on the cholesterol content of bovine milk may be found in part I of the present report [Strzałkowska *et al*. 2009].

Relations reported here between the cholesterol content and the SCC of milk differ from those observed earlier by Strzałkowska *et al*. [2009]. The differences may be attributed to the criteria applied when selecting animals for studies. In the paper cited the animals were selected on the basis of the LGB polymorphic forms, what means that at the time of selection they differed as regards the yield and chemical composition of milk. Moreover, the composition of diets offered to the cows was also different. Thus, in order to obtain reliable results as regards the cholesterol content of cow milk one must consider a range of factors which may affect its concentration.

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Czynniki genetyczne i środowiskowe wpływające na zawartość cholesterolu w mleku krów. II. Wpływ rodzaju stosowanej kiszonki

S t r e s z c z e n i e

Celem pracy było określenie wpływu rodzaju paszy objętościowej stosowanej w żywieniu krów (kiszonka z powiędniętych traw *vs*. kiszonka z całych roślin kukurydzy – odpowiednio grupa 1 i 2) na zawartość cholesterolu w produkowanym mleku. Nie stwierdzono zależności między koncentracją cholesterolu w mleku wyrażoną w mg/100 ml mleka ani w mg/g tłuszczu mleka a rodzajem zadawanej kiszonki. Wykazano natomiast istotną zależność między zawartością cholesterolu w mleku a stadium laktacji, liczbą komórek somatycznych w mleku, dobową wydajnością mleka, zawartością tłuszczu w mleku i ilością tłuszczu wydalanego w mleku w ciągu doby.