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Factors affecting the cholesterol content of milk of cows fed conserved feeds in a TMR system throughout the year

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Summary

The aim of this study was to evaluate changes in the cholesterol content in the milk of high yielding cows fed a uniform diet composed of conserved feeds over the whole year. The investigations were conducted on 124 Polish Holstein-Friesian cows, selected from herd yielding 8457 kg milk with 4,58 % and 3,56 % of fat and protein content, respectively. The cows were maintained in a loose barn and fed *ad libitum* with TMR (total mixed ration) throughout the year. The diets consisted of corn silage and grass silage (at 50:50 ration on dry matter basis) and concentrates with mineral-vitamin mixture additives. Samples of milk were collected individually from each cow at monthly intervals during the whole year. The cholesterol content in milk (mg/dL) and in milk fat (mg/g) was related to the stage of lactation, season of the year, somatic cell count and fat content, but was not affected by the parity. The cholesterol content in daily milk yield (mg/cow/day) depended also on parity. Even though the cows were fed a uniform diet throughout the year according to the TMR system the cholesterol content in milk differed among seasons.

Key words: cholesterol, milk, TMR system, conserved feed, cow

Introduction

The cholesterol content in cow milk is influenced by both genetic and environmental factors.

Despite the fact that the main part of cholesterol contained in milk is synthesised endogenously, via process independent of the offered diet type, the chemical composition of feed (especially fat) affects the content of this sterol in cow milk (Precht, 2001). The content of cholesterol in cow milk is related also to the proportion of somatic cell counts (Strzałkowska et al., 2009a).

In the available literature there are limited information concerning the cholesterol content in milk of cows fed uniform diet composed only of conserved feeds during the whole year, but there are some papers about the cholesterol content in cow blood (Marenjak et al., 2007; Guedon et al., 1999). In light of this, the presented study aimed at evaluating the changes in the cholesterol content in milk of high yielding Polish Holstein-Friesian cows that were fed a uniform diet throughout the year according to the TMR system. The analysis included changes occurring throughout the year and considered the daily milk yield, its chemical composition, stage of lactation, age of cows and the health status of the mammary glands.

Material and methods

Animals

The study was conducted on 124 Polish Holstein-Friesian cows of the Black-and-White variety,

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selected from a herd of 160 head. The mean annual milk yield of cows in that herd amounted to 8457 kg, with a 4.58 % content of fat and 3.56 % content of protein. The cows were maintained in the loose barn and fed *ad libitum* with TMR (total mixed ration) throughout the whole year. Cows were divided into four technological groups, depending on milk yield and stage of lactation. The diets were formulated according to the *Institut National De La Recherche Agronomiuqe* (INRA) standards (2001). The diet consisted of very high quality corn silage (containing 30-35 % dry matter) and wilted grass silage (35 % dry matter), produced with an addition of biological additives. The grass silage was prepared in plastic bags. Both types of silage were mixed in a mash trolley at 1:1 ratio on the dry matter basis. The share of dry matter from silages constituted about 50 % of the total dry matter of the TMR diet. The concentrate mixture was composed of ground grains with an addition of solvent-extracted rapeseed oil meal and a high energy concentrate. The deficiency of mineral elements and vitamins, occurring in the basic ration, was balanced by an addition of a mineral and vitamin supplement. The feed was offered twice daily in amounts ensuring that the leavings would not exceed 5 %. Animals had access to water, which could be heated during the winter months to a temperature of 12-16 °C. Moreover, the cows had *ad libitum* access to salt-lick. Samples of the feed offered were analysed in laboratory and their nutritive value was calculated on the basis of the chemical content, using the computer software INWAR D.J. Group, which renders it possible to evaluate the concentration of PDIN (protein supplied when nitrogen is limited in the rumen), PDIE (protein supplied when energy is limited in the rumen) and FUL (milk fed unit for lactation). Diets were balanced for individual technology groups using computer software (DJ Group s.c., Kraków, Poland).

Chemical analyses

Milk samples (total 1243) for analyses were collected every month during control milking. Samples from the daily milk yield were collected individually from each cow from January 2008 till December 2009. The samples were analysed for total cholesterol, fat, protein, lactose content and the somatic cell count. Fat in the milk samples designated for cholesterol content determination were hydrolysed according to the method by Fletouris et al. (1998). The cholesterol content was determined by the colorimetric method by 490 nm according to Searcy and Berquist (1960) and was expressed as cholesterol content in milk (mg/dL), in milk fat (mg/g) and in daily milk yield (mg/cow/day). The content of fat, protein and lactose was determined using the MilkoScan 104A/B apparatus, while the somatic cell count using the Fossomatic 90 apparatus.

Statistical analysis

The statistical analysis was conducted using the GLM with Bonferoni adjustment and CORR procedures with SAS Version 9.1.3. for Windows (SAS, SAS/STAT 2002-2003). The statistical model included the fixed effects of lactation stage, age of cow, year season, somatic cell count (SCC) and fixed regression for milk yield. Three stages of lactation were identified: 1 - 6[1]-60 days, 2 - 61-210 days and 3 - 211-305[2**] days, as well as three age groups: I - primiparous, II - cows in their second lactation and III - higher than the second lactation. To determine the effect of the somatic cell count 5 classes were separated:

1 - $\leq 10^5$/mL,

2 - $>10^5$ and $\leq 4x10^5$/mL,

3 - $> 4x10^5$ and $\leq 8x10^5$/mL,

4 - $> 8x10^5$ and $\leq 12x10^5$/mL,

5 - $> 12x10^5$/mL.

The four classes of year season were also distinguishing: 1 - winter (January-March), 2 - spring (April-June), 3 - summer (July-September) and 4 - autumn (October-December). Before estimating the Pearson's correlation, the data referring to the somatic cell count were transformed into a natural logarithm.

Ethics

All procedures involving animals were performed in accordance with the Guiding Principals for the Care and Use of Research Animals and were approved by the Local Ethics Commission (Warsaw Agricultural University; Permission No. 56/2009).

1 *Beginning of lactation

2 **End of lactation

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

	Effect				Regression		
	Parity	Season of year	Stage of lactation	Class of SCC	Daily milk yield (kg)	Fat content (%)	Daily fat yield (g/day)
Daily milk yield (kg)	**	**	**	**	—	—	—
FCM# (kg)	**	**	**	**	—	—	—
Total energy in milk (MJ/kg)	**	**	**	ns	—	—	—
Total solids (%)	**	**	**	**	**	—	—
Solids non fat (%)	**	**	**	ns	**	—	—
Fat content (%)	**	**	**	ns	**	—	—
Protein content (%)	**	**	**	**	**	—	—
Lactose content (%)	**	**	*	**	**	—	—
Daily fat yield (g/day)	**	**	**	**	—	—	—
Daily protein yield (g/day)	**	**	**	**	—	—	—
Daily lactose yield (g/day)	**	**	**	**	—	—	—
SCC (ln)	**	ns	*	—	ns	—	—
Cholesterol content expressed as:							
In milk (mg/dL)	ns	**	**	**	ns	**	ns
In daily milk yield (mg/day/cow)	**	**	**	*	—	**	—
In milks fat (mg/g)	ns	**	**	**	ns	**	ns

ns - not significant; **P≤ 0.01; *P≤ 0.05; #FCM - Fat Corrected Milk = 0.4 x milk yield (kg) + 15 x fat yield (kg), SCC - somatic cell count

Results and discussion

The cholesterol contents in milk, expressed as mg/dl milk, mg/g milk fat and mg/day/cow was related to the stage of lactation, season of the year, somatic cell count in milk and fat content (Table 1). The age of cows affected only the cholesterol content expressed as mg/day/cow. According to Sharma et al. (1996) the cholesterol content in cow milk ranged from 8.7 to 25.4 mg/dL of fresh milk and from 2.3 to 3.9 mg/dL of pasteurized milk (Cerutti et al., 1993), whereas calculated per gram of milk fat reported by various authors it ranges from 204 to 381 mg (Precht, 2001; Tomaszewski, 2005). It should be noted that differences observed between the results cited were obtained in experiments without considering all the factors that could affect its content.

The differences in daily milk yield of cows fed a uniform diet throughout the year, according to the TMR system between seasons were observed (Table 2). The daily milk yield during the winter season

Table 2. Least squares means (LSM±SE) for daily milk yield and milk component contents across analyzed factors

Analyzed factor	No. of samples	Daily milk yield (kg)	Total solids (%)	Fat (%)	Total protein (%)	Lactose (%)
Season of year						
Winter	395	19.7 ± 0.2^{A}	13.51 ± 0.01^{A}	4.60 ± 0.01^{Aa}	3.47 ± 0.01^{a}	4.71 ± 0.01^{A}
Spring	398	21.5 ± 0.2^{B}	13.56 ± 0.01^{B}	4.58 ± 0.01^{B}	3.43 ± 0.01^{b}	4.83 ± 0.01^{B}
Summer	411	21.9 ± 0.2^{B}	13.61 ± 0.01^{C}	4.51 ± 0.01^{A}	3.53 ± 0.01^{cd}	4.83 ± 0.01^{B}
Autumn	39	20.1 ± 0.7^{AB}	13.56 ± 0.02^{ABC}	4.54 ± 0.02^{ABb}	3.57 ± 0.03^{d}	4.71 ± 0.04^{A}
Parity						
Primiparous	547	18.2 ± 0.03^{A}	13.56 ± 0.01^{AB}	4.59 ± 0.01^{A}	3.40 ± 0.01^{A}	4.85 ± 0.01^{A}
2nd lactation	366	21.4 ± 0.03^{B}	13.58 ± 0.01^{A}	4.53 ± 0.01^{B}	3.53 ± 0.01^{B}	4.78 ± 0.01^{B}
>2nd lactation	330	22.8 ± 0.03^{C}	13.55 ± 0.01^{B}	4.55 ± 0.01^{B}	3.57 ± 0.01^{B}	4.69 ± 0.01^{C}
Lactation stage (days)						
6-60	257	25.4 ± 0.03^{A}	13.51 ± 0.01^{A}	4.63 ± 0.01^{A}	3.39 ± 0.02^{A}	4.77 ± 0.02^{ab}
61-210	586	21.2 ± 0.03^{B}	13.57 ± 0.01^{B}	4.55 ± 0.01^{B}	3.50 ± 0.01^{B}	4.79 ± 0.01^{a}
211-305	400	15.8 ± 0.03^{C}	13.60 ± 0.01^{B}	4.50 ± 0.01^{C}	3.60 ± 0.01^{C}	4.75 ± 0.02^{b}
SCC/mL milk						
SCC≤10^5	484	21.8 ± 0.03^{A}	13.59 ± 0.01^{A}	4.56 ± 0.01	3.44 ± 0.01^{A}	4.87 ± 0.01^{A}
10^5<SCC≤4x10^5	387	21.4 ± 0.03^{A}	13.58 ± 0.01^{A}	4.55 ± 0.01	3.46 ± 0.01^{A}	4.84 ± 0.01^{A}
4x10^5<SCC≤8x10^5	174	21.4 ± 0.04^{A}	13.57 ± 0.01^{AB}	4.55 ± 0.01	3.50 ± 0.02^{B}	4.78 ± 0.02^{B}
8x10^5<SCC≤12x10^5	100	21.1 ± 0.05^{A}	13.55 ± 0.02^{AB}	4.56 ± 0.02	3.52 ± 0.02^{B}	4.74 ± 0.02^{B}
SCC>12x10^5	98	18.3 ± 0.05^{B}	13.52 ± 0.02^{B}	4.57 ± 0.02	3.57 ± 0.02^{BC}	4.64 ± 0.02^{C}

^{A,B,C}Means within the same column with different superscripts differ (P≤0.01); SCC - somatic cell count

was lower (P<0.01) than during spring and summer. The differences between the mean values did not exceed 2.2 kg/cow/day. However, the differences in the daily milk yield between primiparous and older cows reached 4.6 kg/cow/day while between different lactation stages - 9.6 kg milk/day. The daily milk yield decreased by 3.5 kg/cow when the SCC was higher than 12x10^5 cells/mL of milk.

The differences in the cholesterol contents in the milk expressed as mg/dL and as mg/g of milk fat of cows differing in age proved non-significant (Table 3). The results of the present study do not correspond with those reported by Tomaszewski (2005), who demonstrated that the cholesterol content in milk of cows with a high share of HF genes and fed according to the TMR system increased with the animal age. There were also differences between seasons of the year (P≤0.01); the cholesterol contents were observed in autumn/winter and spring/summer seasons. Similar relations concerning the influence of season on the cholesterol content were reported by Paura et al. (2003) and Tomaszewski (2005).

With the progress of lactation the cholesterol contents expressed as mg/dL milk and as mg/g milk fat increased, while expressed as mg/day/cow decreased. The lower cholesterol content expressed as mg/day/cow was observed at the end of lactation and SCC above 12x10^5. This former relation was confirmed with research conducted by (Tomaszewski and Hubner, 2001) and the latter in earlier

Table 3. Least squares means (LSM±SE) for three measurements of cholesterol content across factors analyzed

Effect	Cholesterol content		
	In milk (mg/dL)	In daily milk yield (mg/day/cow)	In milks fat (mg/g)
Parity			
Primiparous	16.2±0.17	2954.7±54.3^{A}	3.64±0.04
2nd lactation	16.3±0.17	3441.8±56.1^{B}	3.67±0.04
>2nd lactation	16.2±0.17	3614.1±54.2^{C}	3.68±0.04
Season of year			
Winter	15.4±0.14^{A}	2995.5±44.1^{A}	3.49±0.03^{A}
Spring	16.6±0.14^{Ba}	3487.3±45.3^{Ba}	3.76±0.03^{B}
Summer	17.2±0.14^{Cb}	3699.9±45.3^{C}	3.87±0.03^{Ba}
Autumn	15.8±0.41^{A}	3164.8±133.1^{ABb}	3.55±0.10^{ABb}
Lactation stage (days)			
6-60	15.6±0.21^{A}	3883.6±63.7^{A}	3.57±0.05^{A}
61-210	16.3±0.15^{B}	3418.1±47.2^{B}	3.67±0.04^{AB}
211-305	16.9±0.17^{C}	2708.9±54.8^{C}	3.76±0.05^{B}
SCC/mL milk			
SCC≤10^{5}	15.8±0.15^{A}	3395.2±48.7^{A}	3.54±0.04^{Aa}
10^{5}<SCC≤4x10^{5}	16.0±0.15^{A}	3381.2±50.6^{A}	3.61±0.04^{A}
4x10^{5}<SCC≤8x10^{5}	16.1±0.21^{A}	3386.7±69.5^{A}	3.64±0.05^{Ab}
8x10^{5}<SCC≤12x10^{5}	16.4±0.27^{AB}	3427.6±89.1^{A}	3.68±0.07^{AB}
SCC>12x10^{5}	16.9±0.28^{B}	3093.6±89.3^{B}	3.87±0.07^{Ba}

^{A,B,C}Means within the same column with different superscripts differ (P≤0.01)

^{a,b}Means within the same column with different superscripts differ (P≤0.05); SCC - somatic cell count

studies carried out by Strzałkowska et al. (2009a, b).

The cholesterol content expressed as mg/dL of milk was positively correlated with the days-in-milk, total solids, fat, protein and energy content, but negatively correlated with the daily milk yield (Table 4). However, the cholesterol expressed as mg/day/cow in daily milk yield was positively correlated with amount of milk components: fat, protein and lactose expressed in g/day. In turn, cholesterol content expressed in milk fat (mg/g) was negatively correlated with the FCM (Fat Corrected Milk) yield, as well as fat, protein and lactose content.

Analysing the most important factors affecting the content of total cholesterol in the milk of cows with an annual milk yield exceeding 8000 kg milk, under uniform feeding system, the differences in cholesterol content in milk (expressed in mg/dL, mg/day/cow and mg/g) were observed. The seasonal difference between the extreme values amounted to 1.8 mg/dL of milk (11.7 %). A similar variability (0.38 mg/g of milk fat) was recorded for cholesterol content per gram of milk fat (9.8 %). Similar results, at somewhat lower cholesterol content in milk, were observed by Tomaszewski (2005) in cows fed throughout the year according to the TMR system.

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

Traits	Cholesterol content expressed as:		
	In milk (mg/dL)	In daily milk yield (mg/day/cow)	In milks fat (mg/g)
Days in milk	0.31**	-0.48**	ns
Daily milk yield (kg)	-0.27**	0.84**	ns
FCM (kg)	-0.16**	0.83**	-0.24**
Energy of milk (MJ/kg)	0.33**	-0.17**	-0.54**
Total solids (%)	0.33**	-0.21**	-0.50**
Solids non fat (%)	0.27**	-0.32**	-0.20**
Fat (%)	0.30**	ns	-0.59**
Protein (%)	0.31**	-0.37**	-0.16**
Lactose (%)	ns	ns	-0.16**
Fat (g/day)	ns	0.77**	-0.35**
Protein (g/day)	-0.18**	0.81**	ns
Lactose (g/day)	-0.26**	0.83**	ns
SCC (ln)	ns	ns	0.16**
Cholesterol content in milk (mg/dL)	-	0.25**	0.56**
Cholesterol content in daily milk yield (mg/day/cow)	0.26**	-	0.28**

ns - not significant; **P≤0.01;#FCM - Fat Corrected Milk = 0.4 x milk yield (kg) + 15 x fat yield (kg),
SCC - somatic cell count

Conclusions

On the basis of presented study it could be concluded that the cholesterol content in cow milk is related to the stage of lactation, year season and somatic cell count, but is not affected by the parity. In general the cholesterol content in milk of high yielding cows, fed a uniform diet throughout the year according to the TMR system, differed among seasons. It is supposed that apart from this the content of cholesterol is also conditioned probably by the progress of the biochemical processes in the cow organism, controlled by genes which determine its content not only in the cell membranes and blood, but also in the milk. Thus, further research in this area is needed.

Čimbenici koji utječu na sadržaj kolesterola u mlijeku krava hranjenih konzerviranim krmivima TMR sustavom tijekom godine

Sažetak

Cilj ovog istraživanja bio je ispitati promjene udjela kolesterola u visokomliječnih krava hranjenih obrokom sastavljenim od konzerviranih krmiva tijekom cijele godine. Istraživanja su provedena na 124 holstein-frizijske krave, odabrane iz stada koje proizvode 8457 kg mlijeka s 4,58 % mliječne masti i 3,56 % proteina, respektivno. Krave su držane u otvorenom tipu staja i hranjene *ad libitum* s potpuno izmiješanim obrokom (TMR, total mixed ration) tijekom cijele godine. Obrok se sastojao od kukuruzne i travne silaže (50:50, na bazi suhe tvari) i koncentrata s mineralno-vitaminskim dodacima. Uzorci mlijeka prikupljani su pojedinačno od svake krave u mjesečnim intervalima tijekom cijele godine. Sadr-

žaj kolesterola u mlijeku (mg/dL) i mliječnoj masti (mg/g) u vezi je sa stadijem laktacije, godišnjim dobom, brojem somatskih stanica i udjelom masti, ali nije bio pod utjecajem redoslijeda laktacije. Sadržaj kolesterola u dnevnoj količini mlijeka (mg/kravi/dan) ovisi također o redoslijedu laktacije. Unatoč hranidbi krava uniformnom prehranom tijekom cijele godine TMR sustavom, sadržaj kolesterola u mlijeku mijenjao se pod utjecajem sezone.

Ključne riječi: kolesterol, mlijeko, TMR sustav, konzervirana hrana, krava

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