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Studies upon genetic and environmental factors affecting the cholesterol content of cow milk. I. Relationship between the polymorphic form of beta-lactoglobulin, somatic cell count, cow age and stage of lactation and cholesterol content of milk

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Despite the fact that cholesterol is a comparatively stable component of cows' milk its concentration is, within a certain range, subject to significant variation related to the season (probably the feeding system), lactation stage and somatic cell count in milk. The highest differences (about 25%) in the amount of cholesterol per g milk fat were observed between the first and last lactation stage. Despite the decreasing milk yield with the progress of lactation, the amount of cholesterol secreted with milk increased significantly. In the milk of cows for which the somatic cell count was below 100 thousand/ml the cholesterol content was by about 10% lower than that in milk characterized by a higher somatic cell count.

The positive correlation coefficients obtained between the amount of cholesterol expressed as mg/100 ml milk and the per cent of fat and protein indicate that selection conducted for increasing the concentration of nutritive components in milk will result in an increased cholesterol content. However, the quantity of cholesterol per 1 g milk fat will decrease. There was observed no correlation between the content of cholesterol in milk and the polymorphic forms of LGB.

KEY WORDS: dairy cows/ cholesterol/milk /beta-lactoglobulin

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Milk is a common source of protein and fat in the consumer diet. However, one may find opinions suggesting that milk fat, containing a considerable share of saturated fatty acids and cholesterol, has a negative effect on the functioning of the circulatory system, because it may lead to arteriosclerosis [Nath and Murthy 1995]. The greatest fears, in many cases unjustified, roused by the cholesterol contained in milk. The compound is not only a natural milk component, but appears also in the organisms of all humans and animals. It is a precursor of many important steroids, including bile acids, vitamin D, steroid hormones, *etc*. During 24 hours the human organism assimilates 300-500 mg of cholesterol from the diet, while 700-900 mg is created from acetyl-CoA as result of endogenous synthesis [Traczyk *et al*. 2007]. Milk yielded in normal conditions may be an important source of bioactive components such as active peptides, unsaturated fatty acids, fat soluble vitamins, and calcium, easily assimilated by the human organism [Reklewska *et al*. 2003**],** which have a positive effect on the health of consumers. According to Sharma *et al***.** [1996], the concentration of cholesterol in milk varies from 8.7 to 25.4 mg/dl. The differences in the content of this component in milk, reaching as much as 300%, indicate that the concentration of cholesterol depends on many factors. The results of studies conducted on this problem indicate the presence of differences between different cattle breeds [Grega *et al*. 2000; Precht 2001, Šterna and Jemeljanows 2003**]**. In literature one may also find results showing a correlation between the cholesterol content of milk and the age of cows [Tomaszewski and Hibner 2001], stage of lactation **[**Turk *et al*. 2003], physiological condition [Polat and Cetin, 2001] and season [Calamari *et al*. 1999, Paura *et al*. 2003].

In the literature available there were found no results from studies on the relationship between the cholesterol content of milk and milk protein polymorphic forms, the presence of which is related to both milk yield and the content of basic milk components. Among cows with different genetic variants of beta-lactoglobulin (LGB) the highest milk yield was observed in those with genotype LGB AA, while the fat content was the highest in the milk of cows with genotype LGB AB and LGB BB. A majority of the results available refer to the content of cholesterol in the cow's blood, depending on various factors as well as in the total milk sample and the dairy products obtained from it. Until now there are only few studies on the concentration of cholesterol in the milk obtained from individual cows as related to the effect of chosen factors. The results hitherto available vary and it is practically impossible to draw synthetic, reliable conclusions on their basis. Moreover, there is a lack of information on the relationship between protein polymorphic forms and the cholesterol content of milk.

The present study aimed at determining the relations between the concentration of cholesterol in milk of cows throughout the lactation and the polymorphic beta-lactoglobulin forms, seasons (also feeding system), age of cows and stage of lactation.

Material and methods

The study was conducted on 60 milking Black-and-White cows (over 50% of HF blood). Three groups of cows were identified on the basis of the genetic variant of beta-lactoglobulin, *i.e*. LGB AA, AB and BB. The cows were maintained in tied stalls adapted for individual feeding, without litter. During the winter season (200 days) the daily ration consisted of corn silage and grass silage (in a 1:1 weight ratio, calculated on a dry matter basis), concentrate and mineral-and-vitamin supplement. During the summer season (165 days) fresh cut grass was used in place of grass silage. The diets were balanced according to the INRA standards. The feed was offered twice daily. The concentrate was placed in the trough on top of the silage. Animals had permanent access to water in automatic waterers and between feedings spent several hours on a paddock. The cows were milked twice a day at equal 12-hour intervals during the whole lactation. Milk samples were obtained from each cow once a month and analysed for basic chemical composition, somatic cell count and concentration of total cholesterol. On the basis of the milk yield and chemical composition a calculation was made of the daily amount of individual components secreted in milk.

Analytical

The polymorphic forms of beta-lactoglobulin were determined according to the method described by Erhardt [1989]. The content of fat, protein and lactose in milk was determined using a MilkoScan 104A/B apparatus, while the somatic cell count using a Fossomatic apparatus. The extraction of cholesterol from milk and its saponification was conducted according the method described by Fletouris *et al*. [1998], while the quantitative determination of cholesterol was done using the colorimetric method according to Searcy and Berquist [1960].

Statistical

The data were analysed by the SAS General Linear Model procedure [1999].

Two or three cows were the daughters of one sire so the paternal effect was not taken into consideration in the statistical model. Milk samples were divided into three classes depending on the age of the cow (lactation parity): I – cows in their first lactation, II – cows in their second lactation, III – cows in the third or further lactations. Milk samples collected during the whole lactation were also divided into three classes (lactation stages): I – 6-60 day of lactation, II – 61-210 day of lactation and III – between day 211 and end of lactation. The data referring to somatic cell count were transported onto a natural logarithm and four milk classes were separated: I – below 100 thousand/ml, II – 101-400 thousand/ml, III – 401-800 thousand/ml and IV – over 800 thousand/ml. In statistical model were included also season of the year (I – winter, II – summer), polymorphic forms of LGB with regression on milk and fat yield and fat percentage.

Results and discussion

The cholesterol content of milk, expressed both as mg/100 ml milk and as mg/g milk fat, as well as the amount of cholesterol secreted with milk over 24 hours, was significantly (P≤0,001) related to the season, stage of lactation and somatic cell count – Table 1. A significant relation was demonstrated between the daily milk and fat yield and the quantity of cholesterol per g fat and the quantity of cholesterol secreted daily in milk. In turn, there was observed no significant relation between the cholesterol content of milk and the LGB polymorphic form. A significant correlation was observed between the age of cows (lactation parity) and the amount of cholesterol per g milk fat. A higher cholesterol content was recorded during the winter months than during the summer season (Tab. 2). A similar relation was reported by Bernabucci *et al.* [1999] and Calamarie *et al*. [1999], while the results obtained by Kumara and Pachuary [2001] and Paury *et al*. [2003] indicate a reverse trend. The differences between results reported by different authors are difficult to interprete. One may assume that they were caused by differences in the chemical composition of diets offered to the cows. This hypothesis is confirmed by results reported by Tomaszewski *et al*. [2005], who demonstrated that feeding cows according to the TMR system, *i.e*. with a uniform diet throughout the year, results in only small differences in the cholesterol content of milk between seasons. In turn, the results of Cole *et al*. [2001] show a significant relation between the cholesterol content of blood plasma of cows and the type of feeds in their diet. On this basis one may assume that the type of feed

Table 1.The effect of various factors on the cholesterol content in cow milk

Factor	Cholesterol (mg/100 ml of milk)	Cholesterol (mg/day)	Cholesterol (mg/g milk fat)
Season of year	xxx	xxx	xxx
Number of lactation	NS	NS	NS
Stage of lactation	xxx	xxx	xxx
LGB genotype	NS	NS	NS
SCC/ml	xxx	xxx	xxx
Daily milk yield (kg)	NS	xxx	xxx
Fat content of milk (%)	xxx	NS	xxx
Daily yield of milk fat (g)	NS	xxx	xx

xxx – P≤ 0.001; xx– P≤0,001; NS – non significant.

Table 2. Daily milk yield and its composition

Factor	n	Daily milk yield (kg)				Content (%)							
		actual		FCM		total solids		fat		total protein		lactose	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Season of the year													
1	274	12.0	0.4	13.1^{A}	0.1	13.21^{A}	0.08	4.35^{A}	0.06	3.58^{A}	0.03	4.55^{A}	0.02
2	441	12.6	0.3	12.5^{B}	0.1	12.62^{B}	0.07	4.04^{B}	0.05	3.26^{B}	0.03	4.63^{B}	0.02
Number of lactation													
1	75	10.7^{Aa}	0.6	12.8^{a}	0.2	12.97^{AB}	0.13	4.17^{A}	0.09	3.41^{ab}	0.05	4.66^{A}	0.04
2	145	12.0^{Ab}	0.4	12.5^{a}	0.1	12.72^{A}	0.09	4.01^{A}	0.07	3.39^{a}	0.04	4.60^{A}	0.03
3	495	14.0^{B}	0.3	13.1^{b}	0.1	13.08^{B}	0.06	4.40^{B}	0.05	3.46^{b}	0.02	4.50^{B}	0.02
Stage of lactation													
1	98	19.1^{A}	0.5	13.3	0.2	13.09^{B}	0.13	4.43^{a}	0.09	3.41^{a}	0.05	4.53^{A}	0.04
2	522	11.0^{B}	0.3	12.5	0.1	12.54^{B}	0.06	3.98^{b}	0.05	3.29^{b}	0.02	4.56^{A}	0.02
3	95	6.6^{C}	0.5	12.6	0.2	13.13^{A}	0.12	4.16^{c}	0.08	3.55^{c}	0.05	4.68^{B}	0.03
LGB genotype													
1	280	11.7^{a}	0.4	13.0^{A}	0.1	13.1^{A}	0.1	4.28^{A}	0.06	3.42	0.03	4.67^{B}	0.02
2	253	12.5^{b}	0.4	13.0^{A}	0.1	13.0^{A}	0.1	4.30^{A}	0.06	3.42	0.03	4.54^{B}	0.02
3	182	12.6^{b}	0.4	12.4^{B}	0.1	12.7^{B}	0.1	4.00^{B}	0.07	3.41	0.04	4.57^{B}	0.03
SCC/ml													
1	288	13.2^{A}	0.3	13.0	0.1	13.06^{a}	0.07	4.31^{a}	0.05	3.35^{a}	0.03	4.68^{a}	0.02
2	280	11.9^{B}	0.4	12.9	0.1	13.04^{a}	0.08	4.28^{a}	0.06	3.48^{b}	0.03	4.55^{b}	0.02
3	74	11.7^{B}	0.6	12.7	0.2	12.81^{b}	0.13	4.16^{a}	0.09	3.40^{ab}	0.05	4.52^{b}	0.04
4	73	12.3^{AB}	0.6	12.6	0.2	12.77^{b}	0.13	4.01^{b}	0.09	3.43^{ab}	0.05	4.61^{b}	0.04

^{aA}Within traits (columns) means bearing different superscripts differ significantly at: small letters – $P \leq 0.05$; capitals – – $P \leq 0.01$.

offered affected also the content of cholesterol in milk. This hypothesis is confirmed by Hanczakowski [1998], who showed that the cholesterol content decreased in the milk of cows receiving soya protein, while an addition of casein increased the concentration of this component. Precht [2001] demonstrated that a diet for cows containing a high share of rapeseed leads to a decrease of the cholesterol content in milk by 8-13%. In the present studies, despite the lack of significant differences in the amount of cholesterol per 100 g milk between different age groups, animals being in their second lactation yielded milk containing more cholesterol per g milk fat. A similar relation was reported also by Tomaszewski and Hibner [2001]. The greatest effect on the cholesterol content of milk was recorded for stage of lactation. The difference in the concentration of cholesterol in milk, expressed as mg/dl, amounted to about 26% between the first and third stage of lactation. A similar difference was observed when comparing the amount of cholesterol expressed as mg per g milk fat. With the progress of lactation the cholesterol content of milk increased, calculated either per 100 ml milk or per g milk fat. Despite the decrease in milk yield, occurring with the progress of lactation, the amount of cholesterol contained in the daily milk yield increased significantly. Similar observations were reported by Son *et al*. [1996]

Table 3. Cholesterol content of milk as related to various factor

Factor	n	Cholesterol (mg/100 ml of milk		Cholesterol (mg/day)		Cholesterol (mg/g fat)	
		LSM	SE	LSM	SE	LSM	SE
Season of the year							
1	274	18.32^{A}	0.30	2215^{A}	44	4.40^{A}	0.08
2	441	16.95^{B}	0.27	2054^{B}	40	4.10^{B}	0.07
Number of lactation							
1	75	17.53	0.48	2135	69	4.21^{AB}	0.12
2	145	17.98	0.35	2171	50	4.36^{A}	0.09
3	495	17.39	0.24	2098	34	4.19^{B}	0.06
Stage of lactation							
1	98	15.51^{A}	0.48	1762^{A}	69	3.74^{A}	0.12
2	522	17.81^{B}	0.24	2255^{B}	35	4.35^{B}	0.06
3	95	19.58^{C}	0.44	2387^{C}	64	4.66^{C}	0.11
LGB genotype							
1	280	17.66	0.29	2119	43	4.21	0.07
2	253	17.55	0.31	2164	46	4.33	0.08
3	182	17.50	0.35	2121	51	4.22	0.09
SCC/ml							
1	288	16.78	0.27	2051	40	4.02^{A}	0.07
2	280	17.64	0.31	2130	45	4.25^{B}	0.08
3	74	17.90	0.47	2173	68	4.33^{B}	0.12
4	73	18.22	0.46	2184	67	4.41^{B}	0.12

^{ABC}Within traits (columns) means bearing different superscripts differ significantly at $P \leq 0.01$.

and by Turk *et al*. [2003]. Tomaszewski and Hibner [2001] demonstrated that the highest cholesterol content of milk in cows occurs between month seven and ten of lactation. A positive correlation was observed when comparing the content of cholesterol in milk of cows as related to the somatic cell count, though significant differences occurred only between class I milk (SCC below 100 thousand/ml milk) and the remaining classes. The difference in the amount of cholesterol per 1 g fat in milk with a SCC below 100 thousand/ ml compared to milk with a SCC exceeding 800 thousand/ml reached about 10%. The relations presented between the somatic cell count and cholesterol content of milk reflected the changes in the fat concentration in the milk of cows: with the increasing somatic cell count the content of fat in milk decreased (Tab. 3). Despite the significant relation between the polymorphic form of LGB and the daily milk yield and milk fat content (the extreme difference in fat content in milk differing in the LGB polymorphic form reached 0.3 per cent point) no significant correlation was observed between the polymorphic forms of this protein and the cholesterol content of milk. In the literature available the authors did not find results of studies referring to the relations between the health condition of the mammary gland, estimated on the basis of the somatic cell count and the cholesterol content of milk.

Pearson's correlation coefficient between the amount of cholesterol expressed as mg/100 ml milk and the daily milk yield was negative (Tab. 4). In turn, the amount of cholesterol per 100 ml milk was positively correlated with all the milk components; the highest value was obtained for the correlation coefficient with the protein content of milk (r=0.45). A reverse correlation was obtained between the amount of cholesterol per 1 g milk fat. In this case, the correlation coefficient with milk yield was positive and relatively low (r=0.12). The highest absolute value was observed for the correlation coefficient between the amount of cholesterol, expressed as mg/g fat and the per cent fat content of milk (r=-0.44); the correlation coefficient for milk protein content was not significant.

Table 4. Pearson's correlations between cholesterol content, milk yield and its chemical composition

Item	Milk yield (kg)	Total solids (%)	Solids non fat (%)	Fat (%)	Total protein (%)	Total casein (%)	EBM (MJ/kg)	pH
Cholesterol (mg/100 ml)	-0.23xxx	0.42xxx	0.38xxx	0.34xxx	0.45xxx	0.35xxx	0.40xxx	0.17xxx
Cholesterol/fat (mg/g)	0.12xxx	-0.32xxx	-0.02	-0.44xxx	-0.06	-0.12	-0.37xxx	0.22xxx

xxxP≤0.001.

REFERENCES

1. BERNABUCCI U., RONCHI B., LACETERA N., NARDONE A.,1999 – Metabolic status and milk production of Fresian cows during spring and summer periods. Proceeding of the ASPA Congress on Recent Progress in Animal Production Science, Piacenza (Italy) 21-24 June, vol. 1, 452-454.
2. CALAMARI L., MAIANTI M.G., AMENDOLA F., LOMBARDI G., 1999 – On some aspects of the oxidative status and on antioxidants in blood of dairy cows during summer. Proceding of the ASPA Congress on Recent Progress in Animal Production Science, Piacenza (Italy) 21-24 June, 1, 449-451.
3. COLE N. A., BROWN M.A., PHILIPS W.A., 2001 – Genetic x environment interactions on blood constituents of Angus, Brahman and reciprocal-cross cows and calves grazing common bermudagrass or endophyte-infected tall fescue. ***Journal of Animal Science*** 79(5), 1151-1161.
4. ERHARDT G., 1989 – κ- kaseine in Rindermilch – Nachweis eines weiteren Alleles (κ – Cn^{E}) in werschie-denen Rassen. ***Journal of Animal Breeding and Genetics*** 106, 225-231.
5. FLETOURIS D. J., BOTSOGLOU N. A., PSOMAS I. E., MANTIS A. I., 1998 – Rapid determination of cholesterol in milk and milk products by direct saponification and capilary gas chromatography. ***Journal of Dairy Science*** 81, 2833-2840.
6. GREGA T., SADY M., FAROT A., 2000 – Milk fat quality of Simmental breed cows. ***Biotechnology in Animal Husbandry*** 16, 1-2, 49-53**.**
7. HANCZAKOWSKI P., 1998 –Wpływ rodzaju spożytego białka na zawartość cholesterolu we krwi. ***Postępy Nauk Rolniczych*** 45/50, 1, 91-98**.**
8. KUMAR B., PACHAURI S.P., 2001 – Plasma lipids and cholesterol profiles of dairy catlle for monitoring herd health status in the central Himalays**.** ***The Veterinary Record*** 148, 26, 816 – 817.
9. NATH B. S., MURTHY M. K. R., 1995 – Does milk fat causes atherosclerosis ?. Indian ***Journal of Dairy and Bioscience*** 6, 73-77.
10. PAURA L., JONKUS D., KAIRISA D., 2003 – Research on interconnections between fat content and cholesterol level in cow's milk. Information Technologies. Dept. of Control Systems Agronomijas Vestis (Latvia). ***Proceedings in Agronomy*** 5, 232- 235.
11. POLAT U., CETIN M., 2001 – The changes in some biochemical blood parameters during various lactation stages and dry period in dairy cows.***Veteriner Fakultesi Dergisi Uludag Universitesi*** 19 (1-2), 33-39.
12. PRECHT D., 2001 – Cholesterol content in European bovine milk fats. ***Nährung*** 45 (1), 2-8.
13. REKLEWSKA B., BERNATOWICZ E.,2003 – Funkcjonalne składniki mleka – znaczenie dla organizmu oraz możliwości modyfikowania ich zawartości w mleku. ***Zeszyty Naukowe Przeglądu Hodowlanego*** 71, 47-69.
14. SAS, SAS/STAT User's Guide, 1999 – Release 8.E.SAS Institute Inc., Cary, NC, USA.
15. SEARCY R. L., BERGNIST L. M., 1960 – A new color reacion for the quantitation of serum cholesterol. ***Clinical Chimica Acta*** 5, 192.
16. SHARMA K. C., VARINDRA., SUDARSHAN-SINGH., SINGH-S., 1996 – Day to day variations in total cholesterol and some other components of milk cow and buffalo during summer. ***Indian Journal of Dairy Science*** 49, 8, 472-476.
17. ŠTERNA V., JEMELJANOVS A., 2003 – Comparison of fatty acids and cholesterol content in the milk of Latvian cows. ISSN 1392-2130, ***Veterinarija ir Zootechnika*** 22, (44), 95-98.
18. TOMASZEWSKI A., HIBNER A., 2001 – Zawartość cholesterolu w mleku krów rasy czarno-białej (Cholesterol content of milk in Black-and-White Friesian cows) In Polish, summary in English. ***Zeszyty Naukowe AR Wrocław*** 429, 155-161.
19. SON J., GRANT R.J., LARSON L.L., 1996 – Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. ***Journal of Dairy Science,*** 79 (5), 822-830.

20. TRACZYK W.Z., TRZEBSKI A., GODLEWSKI A., 2007 – Fizjologia człowieka z elementami fizjologii stosowanej i klinicznej (Human Physiology with Elements of Applied and Clinical Physiology). In Polish. Warsaw, PWZL.
21. TURK R., JURETIC D., GERES D., TURK N., REKIC B., SVETINA A., 2003 – Serum paroxonase activity in pregnat dairy cows. Procedings of the IV Central European Buiatric Congress, Zagreb, Croatia. April 23-27, 400-405.

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

Mimo, iż cholesterol jest stosunkowo stabilnym składnikiem mleka krów, to jego koncentracja ulega w pewnym zakresie istotnym zmianom w zależności od pory roku (prawdopodobnie związanym z nią systemem żywienia), stadium laktacji i liczby komórek somatycznych w mleku. Największe różnice (ok. 25%) w ilości cholesterolu na g tłuszczu mleka stwierdzono między pierwszym a ostatnim stadium laktacji. Mimo zmniejszającej się wydajności mleka wraz z upływem laktacji ilość cholesterolu wydalanego z mlekiem istotnie wzrastała. W mleku krów z zawartością komórek somatycznych poniżej 100 tys./ml zawartość cholesterolu była o ok. 10% niższa w porównaniu do mleka, w którym ilość komórek somatycznych była wyższa od podanej granicy.

Dodatnie współczynniki korelacji między ilością cholesterolu wyrażoną w mg/100 ml mleka a procentową zawartością tłuszczu i białka wskazują, że selekcja prowadzona w kierunku zwiększenia koncentracji składników odżywczych w mleku będzie pociągała za sobą wzrost koncentracji cholesterolu. Jednakże ilość cholesterolu przypadająca na g tłuszczu mleka będzie zmniejszać się. Nie stwierdzono zależności między zawartością cholesterolu w mleku a polimorficzną formą LGB.