The Effects of Offered Roughage on Cholesterol Levels in the Milk Fat of Cows

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Abstract In order to evaluate the effect of roughage on cholesterol levels in the milk fat of cows fed with three different types of roughage, namely, Italian ryegrass hay, haylage and fresh Italian ryegrass ad lib.. Three experiments respectively were carried out on 6 Holstein dairy cows according to a switch-back trial. Cholesterol levels in the milk fat of cows given fresh grass (261mg/100g) were significantly higher than for those given hay (236), but no significant difference in cholesterol levels was found between haylage feeding (253) and hay feeding (255). The average production of cholesterol in milk during the feeding periods of fresh grass, haylage and hay feedings was 2,498, 2,271 and 2,060 mg/head/day, respectively. There was a large difference between hay feeding and the other two feedings (p<0.01). Serum cholesterol was at a significantly higher level when the cows received hay (167mg/dl) than when the cows received fresh grass (157). Cholesterol levels in the milk fat had no significant relationship to milk fat content or to serum cholesterol level. However, a close relationship was found between cholesterol levels in milk fat and the C4-C14 group content in the milk fatty acids (p<0.01). Considerable individual variations in the cholesterol levels were observed: cows producing high cholesterol milk and cows producing low cholesterol milk were found to exist irrespective of the offered rations. The results indicated that cholesterol levels in the milk fat were influenced by some other factors in addition to the offered ration.


Key words: cholesterol, milk fat, serum, roughage, dairy cows

A significant correlation between high serum cholesterol level and heart disease has been reported in terms of human health1-3), but no relationship between diet and serum cholesterol has been confirmed4,5). However, since cholesterol is evidently the cause of atherosclerosis responsible for heart attacks and strokes, people with high serum cholesterol are generally recommended to limit their cholesterol intake from foods2,3,5).

Recently, cholesterol has been the focus of major interest in Japan because Japanese food consumption patterns have changed to include many meats and dairy products high in cholesterol compared to one or two decades ago. Several investigations6-10) have been made of cholesterol levels in cow's milk and it is known that levels vary according to the breed and other factors such as feed, season and stage of lactation9,10). However, the effect of feed on levels has not been reported in
detail\(^1\)).

The purpose of this paper is to investigate the changes in cholesterol levels in milk after dairy cows have taken three kinds of feed (fresh grass, haylage and hay) prepared from Italian ryegrass (Lolium multiflorum Lam.) and to discuss the relationship between cholesterol levels in the milk and milk fat content, milk fatty acid composition and acetate in the rumen of cows.

Materials and Methods

Animals, diets and samples

Three experiments (Expts 1, 2 and 3) were performed using lactating Holstein cows at the Kyushu Agricultural Experiment Station in March-April and June-July, 1986 and in March-April, 1987.

In all experiments, six cows were kept in a barn from 8:30 a.m. to 8:00 p.m. and in a paddock from 8:00 p.m. to 8:30 a.m.. They were assigned at random to two groups in the sequences of a switch-back trial, each for a 14-day experimental period. All the cows were in mid lactation and not pregnant. Milk samples were collected from each cow for the last 4 days of every two weeks and stored in a deep-freezer (−20°C) until analysis.

In Expts 1 and 2, the cows received Italian ryegrass hay or fresh Italian ryegrass ad lib., in addition to concentrates and beet pulp being equivalent to the nutrient requirements for milk yield. In Expt 2, blood (10 ml) was taken from the jugular vein of each cow into a test tube twice every period, and the serum obtained was stored in a deep-freezer (−20°C) to be analyzed for cholesterol level.

In Expt 3, the cows received Italian ryegrass hay or haylage ad lib., along with concentrates and beet pulp.

DM intake with roughage, milk yield, milk fat percentage, milk fatty acid composition, cholesterol in milk fat and serum cholesterol were determined. DM intake, milk yield, milk fat percentage and milk fatty acid composition in Expts 1, 2 and 3 have already been reported in this Journal\(^{12}\). Serum cholesterol was analyzed using a auto-analyzer (ACL 6000, Olympus Optical Co., Ltd.) via the COD-POD-4 AA method\(^{13}\) at a local clinical analysis laboratory (Higo Rinsho Kensa Kenkyusho Co., Ltd.).

Cholesterol analysis in milk fat

Throughout this study, analytical grade reagents and solvents were used. Cholesterol and β-sitosterol were purchased from Wako Pure Chemical Industries, Ltd. and Tokyo Kasei Kogyo Co., Ltd., respectively.

After being thawed, milk samples were analyzed according to a modified version of the method of Kaneda et al.\(^{14}\). About 30 ml of milk was centrifuged at 1200 × g for 10 minutes. The milk fat obtained (about 800 mg) was taken into a 20-ml glass bottle with a cap, 15 ml of ethyl ether and a small amount of sodium sulfate were added to remove moisture and vigorously shaken to dissolve the fat. After the glass bottle was allowed to stand for 1 h, the ether solution was transferred to a 250-ml flask (tare:
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A), weighed beforehand, and dried up in a desiccator using an aspirator (20 mm Hg) for about 4 h. After the flask containing milk fat (gross: B) was weighed, 40 ml of ethanol and 6.5 ml of 40% pottasium hydroxide were added and the mixture was saponified at 85°C using a Soxhlet extraction apparatus for 1 h. The mixture was transferred to a 100-ml separatory funnel, and 20 ml of water and 1 ml of β-sitosterol solution (100 mg of β-sitosterol was contained in 100 ml of ethyl ether) were added as an internal standard before extracting the mixture with 20 ml of ethyl ether three times. The combined extracts were washed with 20 ml of water three times, passed through a small amount of sodium sulfate, and concentrated to about 2 ml.

The solution containing cholesterol was subjected to gas chromatography with a flame ionization detector (GC-32, Gasukuro Kogyo Inc.), and the chromatograms were obtained on a thermal–pen recorder (Chromatocorder 12, System Instrument Co. Ltd.). A glass column (1.0 m long and 3-mm bore) packed with silicone OV-1, 2% on Uniport HP (60/80 mesh) was used. Usually 2 μl of each sample were injected.

The operating conditions were as follows: column temperature, 250°C; injection port, 260°C; detector, 260°C; H2, 0.8 kg/cm², Air, 1.0 kg/cm². The carrier gas was nitrogen with a pressure of 0.6 kg/cm².

A calibration curve for cholesterol was obtained from the peak area ratios (cholesterol/β-sitosterol) in the gas chromatograms and concentration ratios (cholesterol/β-sitosterol) by using authentic reagents.

The milk fat amount in each flask was calculated as follows; after drying in a desiccator, some of the flasks (tare: A') containing milk fat (gross: B') were dried in a forced-air dryer for 3 h, cooled in a desiccator, and weighed (gross: C). Milk fat amount was defined as (B−A)/(C−A')/(B'−A').

Results and Discussion

Typical gas chromatograms of cholesterol and β-sitosterol are shown in Fig. 1. KANEDA et al. recommended 5-α-cholestane as an internal standard for determining cholesterol, but as shown in Fig. 1, cholesterol, β-sitosterol and campesterol which was derived from impurities in commercial β-sitosterol, were clearly separable on the gas chromatogram. This result indicates that β-sitosterol can be used as an internal standard for determining cholesterol, though the β-sitosterol purchased was contaminated with campesterol. TSUCHIYA et al. reported that β-sitosterol and campesterol were 0.49% and 0.26% of total milk sterol, respectively, but their presence did not affect the determination of cholesterol, because their quantities were negligible and their peaks were not detected in the chromatograms.

DM intake, milk yield, milk fat content, C4 (butyric acid)–C14 (myristic acid) group, C16 (palmitic acid) group and C18 (stearic acid) group content, cholesterol level in milk fat, cholesterol yield and serum cholesterol are shown in Table 1.

DM intake and milk yield increased when cows were fed fresh grass and haylage instead of hay (p < 0.01), and milk fat content reached a higher level when the cows ate haylage in stead of hay (p < 0.05).
Cholesterol levels in the milk fat showed an increase when the cows were offered fresh grass compared to hay \((p<0.01)\), but no significant difference in the cholesterol levels was found between haylage feeding and hay feeding. Large differences in cholesterol production in the milk were observed between hay feeding and the other two feedings \((p<0.01)\). Cholesterol production during the feeding periods of fresh grass, haylage and hay were 2,497, 2,271 and 2,060 mg/head/day, respectively. The mean values for cholesterol in milk fat have been reported to be 400, 281 and 270 mg/100 g by Bachman and Wilcox\(^8\), Tanahashi et al.\(^6\) and Thuchiya et al.\(^7\), respectively. The mean value (248 mg/100 g) obtained in this study is lower than the above three values. According to Bachman and Wilcox\(^8\), cholesterol in milk was partitioned about 83:17 between the milk fat and skim milk phases during the centrifugation of milk. The cholesterol level analyzed in this study is in connection with milk fat and not skim milk. Thus, it does not show the total cholesterol level in milk. Cholesterol levels in milk also appear to be affected by the stage of lactation and by pregnancy;
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**Table 1.** Effects of offered roughage on DM intake, milk yield, milk fat %, cholesterol in milk fat and serum cholesterol

<table>
<thead>
<tr>
<th>Offered roughage</th>
<th>Expt I</th>
<th>Expt II</th>
<th>Expt III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hay</td>
<td>Fresh grass</td>
<td>Hay</td>
</tr>
<tr>
<td>DM intake (kg/day)¹</td>
<td>—</td>
<td>—</td>
<td>3.86</td>
</tr>
<tr>
<td>Milk yield (kg/day)</td>
<td>25.0</td>
<td>29.3**</td>
<td>26.4</td>
</tr>
<tr>
<td>Milk fat (%)</td>
<td>3.39</td>
<td>3.13</td>
<td>3.67</td>
</tr>
<tr>
<td>C4–C14 group (%)</td>
<td>23.10</td>
<td>27.70**</td>
<td>26.53</td>
</tr>
<tr>
<td>C16 group (%)</td>
<td>30.72</td>
<td>30.98</td>
<td>34.50</td>
</tr>
<tr>
<td>C18 group (%)</td>
<td>46.18</td>
<td>41.32**</td>
<td>38.96</td>
</tr>
<tr>
<td>Cholesterol in milk fat (mg/100 g)</td>
<td>235.3</td>
<td>261.4**</td>
<td>230.7</td>
</tr>
<tr>
<td>Cholesterol yield (mg/day)</td>
<td>1967</td>
<td>2376**</td>
<td>2210</td>
</tr>
<tr>
<td>Serum cholesterol (mg/d)²</td>
<td>—</td>
<td>—</td>
<td>167</td>
</tr>
</tbody>
</table>

*: Significant at 5%
**: Significant at 1%
—: Not determined.

¹: DM intake of roughage from 8:30 to 20:00 h.
²: Total cholesterol

Milk cholesterol increased in colostrum⁹ whereas it decreased in milk from cows in late lactation compared to that from cows in mid lactation¹⁰. However, as all the cows in this study were in mid lactation, the cholesterol level seems not to be affected by the above factors.

As shown in Fig. 2, considerable individual variation in cholesterol levels was observed. In the case of cows with high cholesterol levels in milk fat, the cholesterol levels ranged from 263 to 294 mg/100 g depending on the ration, but in low cholesterol cows, the levels ranged from 188 to 230 mg/100 g. The results indicate that cholesterol level in milk fat is influenced by other factors in addition to the offered ration.

As shown in Table 1, contrary to milk fat cholesterol, serum cholesterol decreased when the cows were fed fresh grass compared to hay (p<0.01). The cause of this reversed effect is not clear, but it seems probable that fresh grass lowers cholesterol level in the serum. This would indicate a need to further investigate the effects of fresh grass feeding on serum cholesterol.

In all experiments, there was no relationship between milk fat content and cholesterol level in the milk fat. However, a significant correlation was observed between the C4–C14 fatty acid group and cholesterol levels in the milk fat (p<0.01) and confirmed from the data of an individual cow as demonstrated in Fig. 3. Cholesterol and C4–C14 fatty acids in milk are reported to originate from acetate produced in the rumen¹⁵,¹⁶. The increase of these compounds in milk fat suggests an increase in acetate production in the rumen. This may be also linked to DM intake by the cows vis-a-vis fresh grass and haylage feedings in Table 1.

In the animal's body, cholesterol is synthesized in the liver, the mammary gland and the epithelium of the small intestine. Then, it is partly excreted into milk and
Fig. 2. A relationship between offered roughage and cholesterol levels in milk fat. A to F denote cows' names.

Fig. 3. A relationship between C4-C14 fatty acid group % and cholesterol levels in milk fat. A to F and 1 to 3 denote cows' names and experimental periods, respectively.
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transformed into steroid hormones, cholic acid, etc.\textsuperscript{15-17). According to LONG et al.\textsuperscript{15),}
about 20\% of milk cholesterol is synthesized from acetate in the mammary gland and
80\% is derived from blood cholesterol, suggesting that serum cholesterol is related to
cholesterol levels in milk fat. In this study, a reversed relationship was indicated
between the two factors, but no significant correlation was found.

Milk fat is synthesized in the mammary gland from acetate, butyrate, the higher
fatty acids and glycerol in the blood plasma; in the fatty acids of milk fat
triglycerides, the acids from C\textsubscript{4} to C\textsubscript{14} are mainly synthesized from acetate and
butyrate, while C\textsubscript{16} and C\textsubscript{18} fatty acids are directly incorporated into the
glycerides\textsuperscript{18).}

As shown in Table 1, in most cases milk fat with higher content of the acids from
C\textsubscript{4} to C\textsubscript{14} is also higher in cholesterol. This suggests the possibility of producing
low cholesterol milk by selecting feeds which stimulate the incorporation of long chain
fatty acids into glycerides.

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References

2) HANSEN, R. G., Lactation. vol. III. (LARSON, B.L. and V.R. SMITH, eds.) 281-308.
7) TSUCHIYA, F., Y. YAMAMOTO, T. OKABE and K. AIZAWA, Jpn. J. Zootech. Sci., 43:
14) KANEDA, T., A. NAKAJIMA, K. FUJIMOTO, T. KOBAYASHI, S. KURIYAMA, K. EBIHARA,
17) TERAMOTO, T., M. KINOSHITA and H. Oka, Metabolism and Disease, 25: 339-347.
乳脂肪中のコレステロール濃度に与える給与粗飼料の影響

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給与粗飼料を変えた場合の乳脂肪中のコレステロール濃度を調べるため，各実験毎に6頭のホルスタイン種乳牛を用い，実験I，IIおよびIIIの3回の試験を行なった。乳牛には栄養要求量のうち乳量相当分は配合飼料とヒートパルプで給与し，その他はイタリアンライグラス乾草あるいは同生草（実験I，II）および同乾草あるいは同ヘイレザー（実験III）を自由採取させた。実験計画には各実験とも1期14日間の反転法を用いた。調査項目は，乳脂肪中のコレステロール値および血清中総コレステロール値である。乳脂肪中のコレステロール値は乾草給与（225 mg/100 g）に比べ生草給与（261）では有意に高かったが（p<0.01），ヘイレザー給与時（253）には両者の間に明確な差異が認められなかった。またコレステロールの平均生産量は，生草，ヘイレザーおよび乾草給与時でそれぞれ，2498，2271および2060 mg/頭/日であり，乾草給与と他の二つの給与との間には大きな差異が認められた（p<0.01）。一方血清中総コレステロール値は乾草給与（167 mg/dl）の方が生草給与（157）に比べて高かった（p<0.05）。牛個体別に乳脂肪中のコレステロール値の推移をみると，個体により給与飼料にかかわらずその値が高く推移するものと，反対に低く推移するものが認められた。乳脂肪中のコレステロール値と乳脂肪および血清中総コレステロール値との間には相関関係は認められなかったが，乳脂肪の脂肪酸のC4-C14グループ（％）との間には密接な関係が認められた。以上のこととは，牛乳中のコレステロール濃度は給与飼料以外の要因によっても影響を受けていたことを示唆していた。

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