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Lipid Supplementation of Dairy Cows' Diets: Effects on Milk Fat Composition

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The influence of supplementation of diets with some lipid sources (i.e., whole cottonseeds, flaked whole soybean seeds, and calcium soaps) on the composition of milk fat from dairy cows, bred in 50 farms of the Brescia province, was evaluated; 117 bulk milk samples were collected, and the lipid fraction was analyzed. Considerable variations in the composition of both fatty acids and triglycerides were detected, and these variations showed a good correlation with the amount of crude lipids in diets. The application of ratios between fatty acids as an index for butterfat purity is ineffective due to the modifications of the fatty acid composition.

Keywords: *Fatty acids; butterfat; milk fat; dairy cows*

INTRODUCTION

The use of supplemental fat for the diet of ruminants, especially dairy cows, arises from various reasons, which can be summarized as follows: (1) The energy concentration of diets may be increased. This is particularly interesting since it may reduce the negative energy balance in early lactation and have a positive effect on fertility (Buttler and Elrod, 1991). (2) An adequate energy level can be maintained even when a low dry matter concentration is administered as, for instance, during hot periods (Knapp and Grummer, 1991). (3) The use of energy for milk production is more efficient (Savoini, 1993). (4) Lipid supplementation has a positive effect on the ovarian activity since it increases the plasmatic cholesterol content and, consequently, the precursor of both estradiol and progesterone (Buttler and Elrod, 1991).

However, the use of supplemental fat for diets of dairy cows shows some difficulties since fat, particularly unsaturated fatty acids, reduces the fermentative activity of the ruminal bacteria and protozoa (Palmquist and Jenkins, 1980; Savoini, 1993). Therefore, the diet of cows should be supplemented with either saturated fatty acids or protected lipids, which do not affect ruminal fermentation. Oily seeds, such as cottonseeds and soybean seeds, are a natural source of partially protected fat. In recent years, fatty acid calcium salts have been widely used as protected fat (Bondioli et al., 1989; Ohajuruka et al., 1991).

Lipid supplementation to the diet of dairy cows resulted in an increase in production and showed varying effects on milk quality (Polidori et al., 1986; Bondioli et al., 1989; Savoini et al., 1992; Grummer, 1993; DePeters, 1993). One of these effects is the variation in the acidic composition of milk fat as well as a decrease of the medium-chain fatty acids content and an increase of the long-chain fatty acids content (DePeters, 1993).

This may result in improper evaluations of butter purity (Toppino et al., 1988). This product, as stated by the EEC Regulation (No. 985/68, Article 1), must be obtained exclusively from cow's milk or cream. At present, using ratios between fatty acids, it is difficult to determine whether variations in the acidic composition of milk fat depend on food factors or on possible adulterations, such as addition of foreign fat during butter- or cheesemaking.

The aim of this research was to detect variations in the quality of the lipid fraction of bulk milk as a result of the supplementation of dairy cows' diets with flaked whole soybean seeds, whole cottonseeds, and calcium soaps.

MATERIALS AND METHODS

In order to evaluate the lipid fraction, 117 bulk milk samples from dairy cows bred in 50 farm of the Brescia province and the data on the dry matter intake and on the composition of the corresponding cow's diets were collected a whole year through. Some diets (55%) were supplemented with exogenous fat as flaked whole soybean seeds, whole cottonseeds, calcium soaps, or mixtures of these.

Crude protein, crude lipid, and crude fiber contents were calculated from tables (NRC, 1989) taking into account, for the commercial concentrates, the composition labeled. Fat was extracted from milk by the Rose Gottlieb procedure (FIL-IDF Standard Method, 1987) and analyzed by gas chromatographic methods to evaluate the acidic (Toppino et al., 1982) and triglyceride (Lund, 1988) compositions. This evaluation was carried out to check whether the triglyceride composition was subject to the same changes as the fatty acid composition, due to the presence of fat in the diet.

Statistical Analysis. Data on diet composition were classified according to the presence of supplemental fat in the diet, and the average and standard deviation have been calculated. Multivariate statistical analyses were applied by PARVUS package (Forina et al., 1988) to evaluate the influence of supplemental fat on the composition of the lipid fraction of milk.

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Table 1. Chemical Composition of the Diets

type of added fat	no. of milk samples	dry matter intake (kg/die)		crude protein (%/dry matter)		crude lipid (%/dry matter)		crude fiber (%/dry matter)	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
no fat added	53	17.49	1.69	13.77	1.19	2.67	0.20	21.68	1.56
whole cottonseeds	5	20.19	0.93	15.84	1.07	4.92	0.78	18.91	1.95
flaked whole soybean seeds	6	17.99	0.75	15.32	1.63	3.84	0.65	19.54	0.87
cottonseeds and soybean seeds	40	19.74	1.39	16.54	1.06	5.55	0.69	18.85	1.11
calcium soaps[a]	2	21.31	0.67	15.40	0.45	4.32	1.06	18.06	0.11
soybean seeds and soaps	8	21.14	2.49	16.97	1.45	5.64	1.00	18.33	1.38
cottonseeds, soybean seeds, and soaps[a]	3	20.81	1.37	16.05	0.64	5.68	0.81	17.96	1.21

[a] Average and standard deviation are indicative due to the small number of samples.

The data set included 117 objects (milk samples) and 35 variables (results from the fatty acid and triglyceride compositions). The 117 objects were subdivided into the following two categories: milk from cows fed diets supplemented with exogenous fat (64 fat-supplemented samples) and milk from cows fed diets without exogenous fat (53 control samples).

The 35 variables were subdivided as follows: 12 variables (Table 2) for the fatty acid composition (main acids C4−C18:3 plus the sum of minor acids), 6 variables (Table 2) for the main ratios between fatty acids (R1−R6, corresponding to C14/C12, C18:1/C18, C18/C8, (C6 + C8 + C10 + C12)/C18, C18:2/C8, and C14/C18, respectively), 3 variables (Table 2) for fatty acids classified according to the metabolic pathways for fat synthesis (Grummer, 1991) (S = C4−C14, M = C16−C16:1, L = C18: C18:3), and 14 variables (Table 3) for the triglyceride composition (28−54 carbon atoms). This kind of analysis permits also the evaluation of compounds having an odd number of carbon atoms, but in this research only the triglycerides having an even number of carbon atoms have been taken into account. This data set was subjected to the following statistical analyses.

Data Standardization by Autoscaling (Massart et al., 1988). Autoscaling was applied to all data in order to consider all variables independently of their different numerical value. Hence, all variables had the same weight since they had a mean = 0 and unitary variance.

Principal Component Analysis (Massart et al., 1988). The PCA is an exploratory data analysis, which, through the calculation of the linear combinations of original variables, allows the number of dimensions to be considerably reduced, while maintaining most of the information of the data set, expressed as percent variance.

Linear Discriminant Analysis (Massart et al., 1988). The LDA is a technique used to classify each sample according to the distance (i.e., Mahalanobis distance) from the category centroid. The method was validated by the “leave-one-out” technique, where one object is removed from the data set at a time in order to verify whether this object is correctly classified when the “delimiter” between the two categories is calculated in the absence of the same object. This method allows the classification ability, expressed as prediction ability, to be simulated on an unknown object.

Partial Least Squares (Geladi and Kowalski, 1986). The PLS is a regression technique, which computes latent variables from original variables and can thus be applied to data sets with a large number of highly correlated variables. This change, which is similar to that brought about by the PCA, allows to reduce the dimensionality of data and to obtain uncorrelated factors. Furthermore, the useful information is condensed in the first latent variables and “noise” is kept within the last ones. When PLS was used, the crude lipid content of the diets was added as dependent variable.

Genetic Algorithm for the Selection of Variables (Leardi et al., 1992; Leardi, 1994). This technique was applied to both LDA and PLS.

RESULTS AND DISCUSSION

As shown in Table 1, 53 diets out of 117 were not supplemented with exogenous fat, 62.5% of the remaining 64 diets were supplemented with whole soybean seeds in association with whole cottonseeds, 8% and 9% of the diets were respectively supplemented with cottonseeds and soybean seeds, and 20% of the diets were supplemented with calcium soaps, used alone or in combination with oily seeds. The daily amount added was 0.8−1 kg for soybean seeds, 1.3−1.7 kg for cottonseeds, 1.8−3.6 kg for both oily seeds, and 0.35−0.5 kg for calcium soaps.

As far as the chemical characteristics of the diets are concerned, the crude protein content of the no-fat-added diets is quite low, but this is the value registered in this survey. The increased crude protein and the lower crude fiber content of the diets supplemented with fat can be explained by the higher requirements for proteins of the cows fed these diets.

The analysis of the percent composition of fatty acids (Table 2) exhibits both a decrease in the mammary synthesis of short- and medium-chain fatty acids for cows fed diets supplemented with fat and a higher amount of long-chain fatty acids from blood. This confirms data obtained by various authors, who used both protected and unprotected animal and vegetable fat (Banks et al., 1984; Chilliard et al., 1986; Salimei et al., 1992).

The analysis of individual fatty acids showed high oleic (18−20% higher than in the control) and linoleic acid contents in milk fat from cows fed diets containing exogenous fat, as compared to cows fed nonsupplemented diets. In particular, a high linoleic acid content was observed for diets supplemented with whole soybean seeds in association with cottonseeds (3.15%) and calcium soaps (3.29%).

The ratios between fatty acids commonly used to evaluate butter purity (Toppino et al., 1982) showed considerable variations as a result of the change in the acidic composition of fat. In particular, values for the ratios R3 (C18:0/C8), R4 (C6 + C8 + C10 + C12)/C18: 0), and R6 (C14/C18:0) exceeded usual genuineness indices (R3 = <7.63, R4 = >0.95, R6 = >1.02).

Finally, Table 3 shows the results of the triglyceride composition. Data confirm that supplementation of diets with fat affects milk fat composition. In particular, a higher C54 content, which results from the combination of three fatty acids with 18 carbon atoms, was observed in the diets supplemented with fat than in the controls. A 43% increase of the C54 content was found for diets supplemented with calcium soaps up to an increase above 100% for diets containing soaps, soybean seeds, and whole cottonseeds.

Figure 1 shows the plot of the loadings of the variables on the first and second principal components, which respectively explain 58.7% and 10.2% of the total variance of the 35 original variables. Some variables almost coincide in the bidimensional plot, i.e., they have

Table 2. Fatty Acid Composition of Milks Classified According to the Cows Feeding, Expressed as Percent of Major Acids, Ratios, and Different Chain Lengths

variable no.	carbon atoms	control (53)		cottonseeds (5)		soybean seeds (6)		cottonseeds + soybean seeds (40)		calcium soaps (2)[a]		calcium soaps + soybean seeds (8)		cottonseeds + soybean seeds + calcium soaps (3)[a]	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
15	C4	4.30	0.85	4.25	0.56	4.77	0.77	4.18	0.90	4.22	0.36	4.55	0.31	4.12	0.58
16	C6	2.39	0.29	2.31	0.33	2.33	0.15	2.21	0.25	2.13	0.06	2.24	0.22	2.05	0.27
17	C8	1.49	0.23	1.38	0.19	1.39	0.24	1.35	0.20	1.21	0.09	1.21	0.13	1.19	0.26
18	C10	2.90	0.36	2.46	0.18	2.61	0.16	2.50	0.32	2.57	0.42	2.44	0.33	2.21	0.46
19	C12	3.34	0.42	2.72	0.24	2.93	0.21	2.79	0.37	2.97	0.56	2.76	0.33	2.50	0.53
20	C14	11.57	0.83	9.64	0.76	10.17	0.67	9.91	0.92	10.14	1.37	9.60	0.72	9.23	1.77
21	C16	29.33	1.52	26.81	2.29	26.96	1.41	26.29	1.80	28.11	0.34	27.07	1.20	28.06	1.22
22	C18	9.86	0.91	12.83	2.08	11.19	1.28	13.26	1.63	10.26	0.94	11.87	0.77	12.61	1.71
23	C18:1	22.29	1.87	26.01	2.17	25.73	1.32	26.30	1.97	26.44	1.78	26.56	1.52	27.51	2.05
24	C18:2	2.23	0.39	2.78	0.64	2.65	0.59	3.15	0.58	2.85	0.06	3.29	0.37	2.79	0.04
25	C18:3	1.01	0.21	0.81	0.10	0.99	0.24	0.99	0.22	1.00	0.23	1.14	0.21	1.12	0.20
26	others	9.20	0.98	7.93	1.50	8.21	0.85	7.05	0.86	8.02	0.53	7.20	0.28	6.63	0.83
27	R1	3.50	0.31	3.53	0.17	3.48	0.07	3.57	0.23	3.43	0.18	3.50	0.18	3.72	0.32
28	R2	2.27	0.17	2.05	0.19	2.32	0.20	2.00	0.19	2.59	0.06	2.24	0.12	2.19	0.15
29	R3	6.80	1.50	9.53	2.61	8.13	0.71	10.14	2.46	8.51	1.44	9.91	1.62	11.25	4.31
30	R4	1.04	0.19	0.71	0.16	0.84	0.09	0.69	0.16	0.88	0.19	0.73	0.11	0.65	0.19
31	R5	1.52	0.36	2.03	0.50	1.92	0.42	2.40	0.62	2.36	0.23	2.73	0.38	2.44	0.62
32	R6	1.19	0.17	0.77	0.20	0.92	0.14	0.77	0.16	1.00	0.23	0.81	0.10	0.75	0.22
33	S	26.69	2.09	23.23	0.76	24.78	1.01	23.44	2.22	23.82	2.29	23.29	1.92	21.72	3.23
34	M	32.47	1.59	29.73	2.63	29.92	1.59	28.95	1.89	31.19	0.25	29.83	1.31	30.93	1.11
35	L	36.93	2.89	43.86	3.82	41.88	2.73	44.81	3.84	41.77	3.01	43.93	2.28	45.18	3.77

[a] Average and standard deviation are indicative due to the small number of samples. Numbers in parentheses indicate the number of samples for each group.

Table 3. Triglyceride Composition of Milks Classified According to the Cows Feeding

variable no.	carbon atoms	control (53)		cottonseeds (5)		soybean seeds (6)		cottonseeds + soybean seeds (40)		calcium soaps (2)[a]		calcium soaps + soybean seeds (8)		cottonseeds + soybean seeds + calcium soaps (3)[a]	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
1	C28	0.61	0.12	0.50	0.10	0.62	0.08	0.53	0.08	0.57	0.11	0.55	0.11	0.48	0.24
2	C30	1.19	0.18	1.00	0.11	1.17	0.09	0.99	0.16	1.09	0.24	1.07	0.18	0.94	0.24
3	C32	2.47	0.33	2.07	0.14	2.13	0.31	1.92	0.29	2.21	0.40	1.99	0.37	1.78	0.34
4	C34	5.86	0.64	4.69	0.50	5.10	0.34	4.49	0.63	4.86	0.50	4.67	0.52	3.78	0.72
5	C36	11.04	1.12	9.67	1.05	9.72	0.49	9.11	0.85	9.63	0.76	9.25	0.63	8.78	0.53
6	C38	13.30	0.68	12.86	1.75	13.31	0.99	12.89	0.63	12.97	0.72	13.35	0.68	13.25	1.06
7	C40	10.45	0.49	11.57	0.65	11.25	0.60	11.22	0.81	10.99	0.33	11.20	0.65	10.53	0.42
8	C42	7.33	0.56	6.54	0.59	6.82	0.33	6.51	0.45	6.86	0.66	6.45	0.51	5.84	0.82
9	C44	6.84	0.71	5.88	0.30	6.11	0.30	5.59	0.59	6.09	0.86	5.64	0.46	5.08	1.00
10	C46	7.59	0.54	6.61	0.27	6.81	0.34	6.36	0.56	6.77	0.87	6.39	0.52	5.79	1.18
11	C48	9.14	0.50	8.59	0.34	8.67	0.45	8.23	0.58	8.69	0.44	8.47	0.33	8.11	0.93
12	C50	11.07	0.91	11.91	0.63	10.86	1.40	11.85	0.69	11.92	0.76	12.02	0.85	12.62	0.60
13	C52	9.47	1.68	11.98	1.58	11.85	1.30	13.07	1.42	12.11	2.51	12.69	1.73	15.42	2.68
14	C54	3.64	0.85	6.10	1.58	5.57	0.76	7.22	1.68	5.24	1.19	6.26	1.08	7.61	1.29

[a] Average and standard deviation are indicative due to the small number of samples. Numbers in parentheses indicate the number of samples for each group.

the same loading on both components. This indicates, as was expected, that they provide the same information.

Figure 2 shows the plot of the scores of the 117 milk samples subdivided into the following two categories: control (0) and samples supplemented with fat (labeled from 1 to 6 according to the lipid supplementation). The controls tend to separate from the samples supplemented with fat in the left region of the plot. The separation between the two categories occurs on the first-component axis. No distinction is observed between the different lipid supplementations. This result demonstrates that the main constituents of the lipid fraction of milk are largely influenced by supplementation independently of the kind of fat added.

The two categories, i.e., control and fat-supplemented samples, have similar variance−covariance matrices, shown by the fact that they have similar data scattering and orientation with respect to the principal components. Therefore, the linear discriminant analysis was used as a classification technique.

Results from LDA are reported in Table 4, where data for 35 variables, i.e., fatty acids and triglycerides, allow us to obtain an optimal classification, expressed as percent objects correctly assigned to their category. Validation results, though slightly lower than the classification ability, are satisfactory.

The variables were selected by a genetic algorithm applied to LDA in order to remove the variables which provided either the same information or nondiscriminant information. The linear discriminant analysis is particularly suitable for data sets where the number of objects is higher than the number of variables plus the number of categories. The seven variables thus selected are as follows: three triglyceride groups (C40, C42, and C52), linoleic acid (C18:3), the sum of minor acids (others), the ratio C18:2/C8 (R5), and the sum of acids from C4 to C14 (S). The classification ability was similar to that obtained from the complete data set, but a much better prediction ability was observed. This result demonstrates that the most discriminating variables were selected and that the information obtained

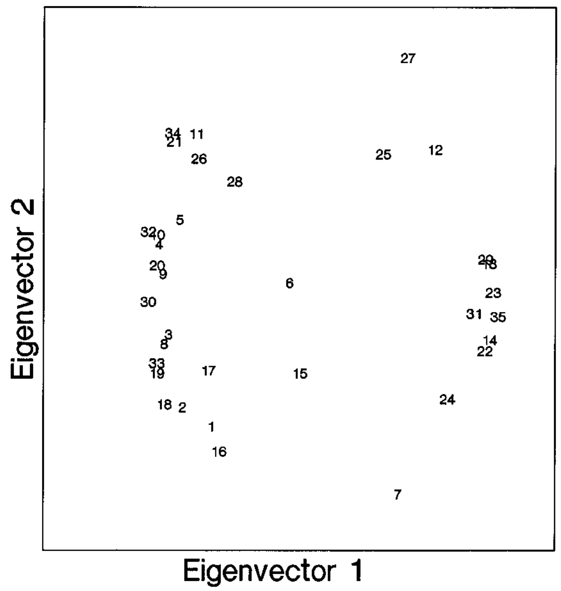

Figure 1. Plot of the loadings of the 35 variables on the first and second principal components (the numbering is the same as in Tables 2 and 3).

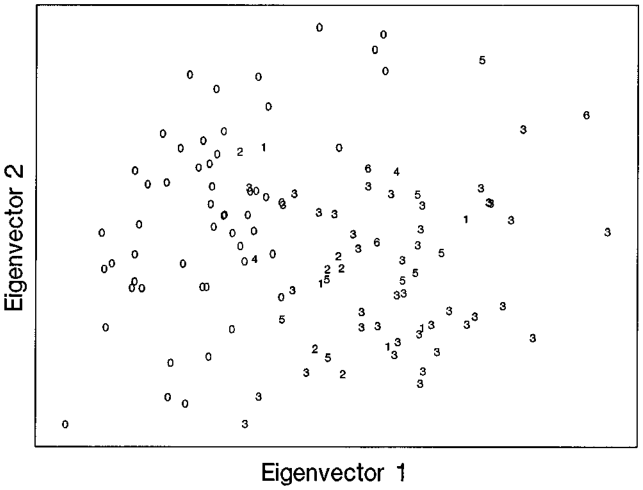

Figure 2. Plot of the scores of the 117 milk samples on the first and second principal components: (0) control, (1) cotton, (2) soybean, (3) cotton + soybean, (4) soaps, (5) soaps + soybean, and (6) cotton + soybean + soaps.

Table 4. LDA Results

	classification ability (%)		prediction ability (%)	
	35 variables	7 variables	35 variables	7 variables
total	97.3	95.9	87.0	95.0
control category	96.2	98.1	84.9	96.3
fat category	98.4	93.7	89.1	93.7

from acids and triglycerides may be considered complementary in the detection of variations in the lipid composition of milk due to the lipid supplementation of diets.

In a following step, the PLS regression technique was applied with the goal of predicting the crude lipid content of the diet from the 35 variables describing the milk fat composition. For this scope the content of crude lipid of diets was considered as the dependent variable. When PLS was applied, with five deletion groups, two significant components were detected; the quality of the prediction was rather good since 70.6% of variance has been predicted, with a residual mean square error in prediction (RMSEP) of 0.80. Three outliers (indicated by a square) can be detected by examining Figure 3, where the crude lipid content of the 117 samples is compared to that predicted by PLS. These samples showed high crude lipid contents (7.54, 7.12, 6.38). Removal of these three samples increases the percent explained variance up to 79.2% (RMSEP, 0.65) with four components (Figure 4).

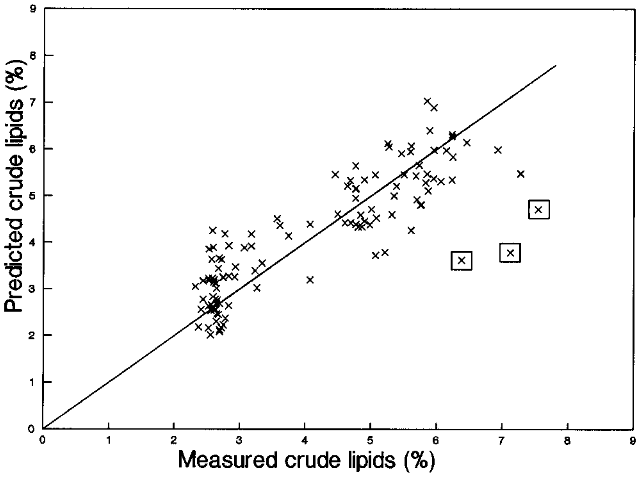

Figure 3. Plot of the results from PLS on 117 samples (crude lipid content versus predicted lipid content).

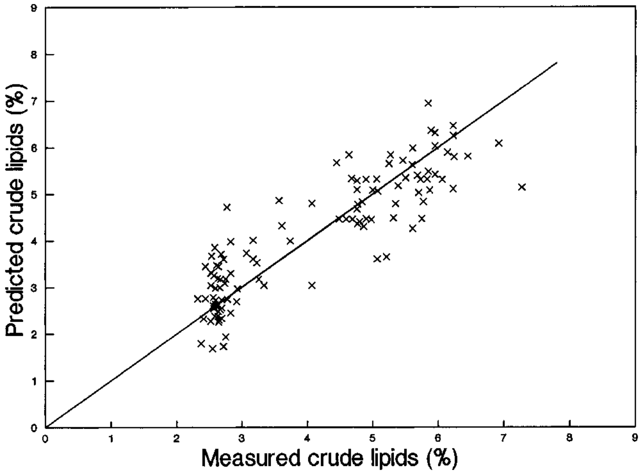

Figure 4. Plot of the results from PLS after removal of three outliers (crude lipid content versus predicted lipid content).

The results obtained by LDA and PLS techniques suggest that the composition of the lipid fraction of milk can be used to determine whether the diet is supplemented with fat as well as the crude lipid content of the cows' diet.

The genetic algorithm was also applied to PLS in order to verify whether the prediction ability could be improved by selecting some variables as for LDA. The selection provided results similar to those obtained from the complete data set. The variables were also selected from both the triglyceride and fatty acid groups; as a consequence they did not reduce the number of analyses. The data were then subdivided into two separate data sets for fatty acids and triglycerides. The application of PLS to the two separate sets provided similar results, which were, however, slightly lower than those obtained from all variables. The percent prediction variance was 73.2% for triglycerides with three components and 72.9% for fatty acids with five components. Therefore, being satisfactory also the results obtained by only one of the two analyses, the expert will decide whether the information fraction is worth the work and the cost of both analyses.

CONCLUSIONS

It may be concluded that administration of exogenous fat to dairy cows produces considerable changes in the lipid constituents of milk. Moreover, the changes detected in the acidic and triglyceride composition showed a high correlation with the amount of crude lipids in the diet.

These results demonstrate that acidic ratios cannot be used as purity indices for butter made from milk from cows fed whole soybean and cottonseeds, soaps, or mixtures of these components. If they were used, a pure butter produced from milk deriving from cows fed a lipid supplemented diet could be considered as a mixture of milk and nonmilk fat. On the other hand, the high correlation between the composition of the lipid fraction of milk and the crude lipid content of the diet may be useful to detect whether milk is from cows fed fat-supplemented diets and to evaluate the crude lipid content of diets.

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