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Conjugated Linoleic Acid (CLA) Content of Milk from Cows Fed Different Diets

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Conjugated linoleic acid (CLA) is a naturally occurring fatty acid in foods derived from ruminants. CLA is a term used for a mixture of positional and geometric isomers of linoleic acid that contain conjugated unsaturated double bonds. CLA has been shown to have many properties that are beneficial for human health. Two experiments were conducted at the same time on two groups of midlactating primiparous cows to determine the conjugated linoleic acid content of milk. Experimental diets were fed for 13 months – the first group of animals was offered a maize silage-based diet while the second group was fed a grass hay-based diet. Grass hay based diet supplied a significantly ($P<0.01$) larger CLA concentration in every controlled month. The CLA content in cows fed a grass hay-based diet varied between 153.85 mg/kg of milk reported in May 2004 and 419.01 mg/kg of milk reported in July 2004. The CLA content in group fed maize silage-based diet varied between 150.58 mg/kg of milk reported in May 2004 and 303.05 mg/kg of milk reported in July 2004. Increasing the CLA concentration in milk can increase the nutritive and therapeutic value of milk and dairy products.

Key words: Maize silage, grass hay, dairy cows, milk, conjugated linoleic acid.

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Continuous interest on conjugated linoleic acid is attributed to its potential health benefits such as anticarcinogenic, antiatherogenic, antidiabetic and antiadipogenic effects. Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12 $C_{18:2}$). Conjugated linoleic acid occurs naturally in foods, however, the main dietary sources are dairy products and other foods derived from ruminants (DHIMAN *et al*. 1999; BESSA *et al*. 2000; STASZAK *et al*. 2001).

REKLEWSKA and BERNATOWICZ (2002) reported that the CLA content in bovine milk varies between 0.3 and 1.4% of the sum of milk fatty acids.

CLA (mostly *cis*-9, *trans*-11 $C_{18:2}$) is formed in the rumen as an intermediate of microbial hydrogenation of dietary linoleic acid and also in the animal tissues from *trans*-11 $C_{18:1}$ (vaccenic acid) also arising from incomplete biohydrogenation in the rumen. Many dietary factors affect the levels and isomeric proportions of CLAs in bovine milk (BAUMAN *et al*. 2001; STASZAK *et al*. 2001; OFFER 2002). These include the amount and type of dietary fat consumed and the proportions of starch and fibre in the diet (OFFER 2002). The CLA concentration can be positively influenced by animal diet. Grazing cows on pasture, feeding fresh cut pasture, addition of fish oil etc. demonstrate positive effects on CLA content in milk (BESSA *et al*. 2000; STASZAK *et al*. 2001).

Typical consumption of CLA by humans is far lower than the dose that has been shown to be effective in reducing tumors in animal models (DHIMAN *et al*. 1999), so it is very important to increase the CLA content in edible products derived from ruminants.

Several surveys have shown that milk fat CLA concentrations are higher for summer milk produced from cows grazing on fresh pasture than for winter milk when conserved forages are fed (JAHREIS *et al*. 1997). However, such studies have usually been confounded by the effects of stage of

The study was conducted with the permission of the Regional Ethical Committee in Bydgoszcz.

lactation and the use of different supplements (OFFER 2002).

The objective of this research was to determine the CLA content of milk from cows fed diets containing different proportions of conserved forages.

Material and Methods

The experiment was conducted from July 2003 to July 2004. Twenty eight primiparous Polish black and white cows with 86% Holstein-Friesian upgrade in midlactation were used.

At the start of the experiment cows averaged 165.78 DIM (SD=88.10) and were yielding 20.15 kg/d of milk (SD was 4.94). Cows were weighed at the beginning of the study; average body weight of the animals was 550 kg (SD=35kg).

Cows were randomly assigned to one of two groups which consumed diets containing different proportions of conserved forages. Forages were conserved the previous summer without additives in normal environmental condition. Group S was fed a diet based on maize silage (50% of dry matter intake) and group H was offered a diet based on grass hay (50% of dry matter intake). During the summer months both diets contained 15-25% fresh cut grass.

The chemical composition of feeds was determined and nutritive value was calculated from chemical composition according to INRA (1988).

Diets were formulated to meet the nutrient demand of lactating cows according to INRA (1988) recommendations. The NDF and ADF contents of the diets were within the recommended ranges for lactating cows. Cows in both groups were given the same mineral-vitamin supplements during the whole experiment.

The ingredient composition of the diets is given in tables 1 and 1a.

Table 1

The ingredient composition of the group fed maize silage-based experimental diets

Feed	Summer diet		Fall diet		Winter diet		Spring diet		Next summer diet	
	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)
Concentrate*	2.5	2.23	4.5	4.02	4.5	3.99	4.5	4.01	2.5	2.20
Maize silage	5.0	1.7	14	4.4	9.5	3.7	14.0	4.6	7.0	3.5
Grass hay	10.3	8.3	9.7	8.2	10.1	8.5	9.5	8.7	9.7	8.9
Sugar beet leafs silage	---	---	---	---	4.0	1.1	---	---	---	---
Alfalfa green forage	28.1	4.3	---	---	---	---	---	---	17.6	3.1
Total	45.9	16.5	28.2	16.6	28.1	17.3	28.0	17.3	36.8	17.7

* concentrate dose for lactating cow, weighting 550 kg, with daily milk production of 19 kg of 4.5% milk fat and 3.4% milk protein

Table 1a

The ingredient composition of the group fed grass hay-based experimental diets

Feed	Summer diet		Fall diet		Winter diet		Spring diet		Next summer diet	
	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)	Fresh matter (kg)	Dry matter (kg)
Concentrate*	2.5	2.23	4.5	4.02	4.5	3.99	4.5	4.01	2.5	2.20
Maize silage	23.9	8.3	26.6	8.4	22.9	8.9	26.2	8.5	19.0	9.5
Grass hay	2.4	1.9	5.0	4.2	4.5	3.8	4.8	4.4	4.5	4.1
Sugar beet leafs silage	---	---	---	---	5.0	1.4	---	---	---	---
Alfalfa green forage	27	4.1	---	---	---	---	---	---	17.9	3.2
Total	55.8	16.6	36.1	16.6	36.9	18.0	35.5	17.0	43.9	19.0

* concentrate dose for lactating cow, weighting 550 kg, with daily milk production of 19 kg of 4.5% milk fat and 3.4% milk protein

Table 2

Conjugated linoleic acid content in bovine milk (mg/kg of milk)

		The CLA content in milk (mg/kg of milk)						
		July 2003	September 2003	November 2003	January 2004	March 2004	May 2004	July 2004
Group S	Mean	184.10[A]	210.06[B]	213.16[C]	177.83[D]	200.29[E]	150.58[F]	303.05[G]
	SD	60.83	86.79	46.74	39.93	106.04	70.91	120.61
	Vx	33.04	41.31	21.92	22.45	52.94	47.09	39.79
Group H	Mean	346.46[A]	283.49[B]	305.21[C]	182.13[D]	206.16[E]	153.85[F]	419.01[G]
	SD	198.55	178.71	116.03	43.10	75.83	63.82	176.99
	Vx	57.31	63.03	38.01	23.66	36.78	41.48	42.24

[A,B,C…] means in the same column with the same superscripts differ significantly at $P<0.01$

Table 3

Characteristics of some milk performance traits in experimental groups (values represent means of total time of experiment)

	Group S		Group H	
Variable	Mean	SD	Mean	SD
Milk yield (kg/day)	20.07	7.18	19.03	6.80
CLA (mg/kg of milk)	204.19	88.93	278.44	161.58
Total solids (%)	12.99	1.06	13.50	1.15
Fat (%)	4.15	0.71	4.48	0.70
Protein (%)	3.38	0.39	3.49	0.50
Lactose (%)	4.53	0.22	4.50	0.21
Somatic cells count (thous/l)	613.59	1079.00	671.41	1608.88
Lnscc	12.63	1.08	12.55	1.06
Lnscc2	4.62	1.56	4.50	1.53
Milk urea (ml/l)	195.65	107.24	216.24	86.11
Days in milk	198.77	119.64	241.29	128.14

Cows were housed in a tie-stall barn and were fed and milked twice daily.

Samples of milk from individual cows were collected monthly and analysed for chemical composition, including the CLA content (analysed every two month).

Daily milk yields were recorded.

Statistical evaluation was made by analysis of variance with the Tukey test for all groups (SAS, 1995). The differences were considered significant at $P<0.05$ and $P<0.01$.

Results and Discussion

The average CLA content in milk analysed during particular experimental milkings is shown in Table 2. The CLA content varied between individual cows which suggests animal related factors.

Milk yield and chemical composition observed during the experiment was similar for both groups (Table 3).

The grass hay based diet supplied a significantly ($P<0.01$) larger CLA concentration in every controlled milking. Diets containing all grass hay supply large amounts of $C_{18:3}$ and small amounts of $C_{18:1}$ fatty acid (DHIMAN *et al*. 1999).

The CLA content within cows fed a grass hay-based diet varied between 153.85 mg/kg of milk reported in May 2004 and 419.01 mg/kg of milk reported in July 2004.

The CLA content in the group fed a maize silage-based diet varied between 150.58 mg/kg of milk reported in May 2004 and 303.05 mg/kg of milk reported in July 2004.

The lowest CLA concentration was determined in both groups in May 2004, which probably was the result of feeding conserved forages which were stored for a long time (forage was produced during the previous summer).

In the summer months of 2003 and 2004 both diets fed in the present study contained fresh cut-grass , which resulted in an increase of CLA levels in milk in both groups. As reported by DHIMAN *et al*. (1999), increasing the proportion of grazed grass from pasture in the diet of dairy cows linearly increases the CLA content of milk.

PATKOWSKA-SOKOŁA *et al*. (2000) report the average CLA content in bovine milk as 33.27 mg/100 g of milk.

In a study conducted by DHIMAN *et al.* (1999), conjugated linoleic acid was 3.8 mg/g of milk fatty acids when a maize silage based diet was fed and 3.9 mg/g of milk fatty acid when a high oil maize silage based diet was fed. When cows were fed either 66.6% grass hay and 33.4% grain, or 98.2% grass hay the CLA concentration was 9.0 and 7.9 mg/g of milk fatty acids, respectively (DHIMAN *et al*. 1999).

DHIMAN *et al*. (1999) observed that cows grazing on pastures and receiving no supplemental feed had 500% more conjugated linoleic acid in milk fat than cows fed typical dairy diets (TMR containing conserved forage and grain in a 50:50 ratio), however, the CLA content in milk remained unaffected when grass from neighbouring paddocks was harvested and fed as hay.

Levels of CLA in the study reported by OFFER (2002) were much higher in the milk fat from cows grazing on fresh grass than from those consuming silage-based diets.

The CLA in milk originates from the rumen and is formed as an intermediate in the biohydrogenation process of unsaturated fatty acids.

Fatty acid composition in fresh and conserved forages were reported by DHIMAN *et al*. (1995) – pasture grass contained an average of 34.0 mg/g of fat $C_{18:1}$, 159.1 mg/g fat of $C_{18:2}$ and 410.8 mg/g of fat $C_{18:3}$; maize silage – 212.8, 487.8 and 41.6 mg/g of fat $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ respectively, alfalfa hay contained approximately 26.1, 150.2, 230.2 mg/g fat of $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ respectively.

Green pasture may contain up to 3% fatty acids on a dry matter basis, of which about 90% are unsaturated C_{18} acids (BESSA *et al*. 2000). KAY *et al*. (2004) reported that the lipids in pasture are high in linolenic acid and rumen biohydrogenation of linolenic acid does not produce cis-9, trans-11 CLA as an intermediate. Results obtained by KAY *et al*. (2004) show that endogenous synthesis is responsible for more than 91% of the cis-9, trans-11 CLA secreted in milk fat of cows fed fresh pasture.

Higher levels of CLA in cows fed grass hay based diets in the present study may be the effect of the lipid composition of the preserved forage, which remains relatively unchanged from that prior to preservation unless there is gross deterioration (during normal drying and storing the $C_{18:3}$ content decreases and $C_{16:0}$ increases in forage, while during normal ensiling and storing $C_{18:3}$ content decreases more significantly) (DHIMAN *et al*. 1995; DHIMAN *et al*. 1999).

Conclusions

Results from the present research show that CLA content of cows' milk fat can be increased through different nutritional practices.

The method of forage preservation differentiated the CLA content in milk. Levels of CLA acid were much higher in the milk fat from cows fed a grass hay-based diet than from those consuming silage-based diets. Higher levels of CLA were noted during the summer months of the experiment within both groups when cows were offered cut grass.

Increasing the CLA content of milk has the potential of increasing the nutritive and therapeutic value of milk.

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