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D2A.1.4 Report of the effect of feedstuff supplementation with linseeds on milk tocopherol and unsaturated fatty acid content

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SUMMARY

The effect of feedstuff supplementation with linseeds on incidence of mastitis and milk tocopherol and unsaturated fatty acid content was studied on 20 Friesian dairy cows, in an experimental farm and for a period of 11 months. The effect of feedstuff supplementation with linseeds was correlated to a small reduction in somatic cell counts; anyhow, this result was of no significant interest to minimize mastitis incidence without the use of synthetic vitamin E supplementation. In the whole, the supplementation of linseeds proved useful to raise the global milk production. In parallel, it showed also very positive effects on the general welfare of the animals and on the milking process through the improvement of the milk flows. On the other side, a significant higher percentage of lactose in the milk was found. Significant higher quantities of protein, fat, lactose and PUFA were produced for each milking.

INTRODUCTION

Healthy cows are the foundation for sustainable milk production. However, mastitis and other infectious diseases are common problems in dairy herds, resulting in increased costs and decreased production. Most diseases in dairy cows occur at or just after calving, which is a period associated with immune suppression, resulting in an increased susceptibility to infections. Several different ingredients of feedstuff may be able to mitigate the severity of the clinical symptoms of mastitis and to shorten their effects by strengthening the udder defence mechanisms. The nutrition program of a dairy herd has a major influence on cow productivity and health.

Nutritionists correlate several severe human pathologies (cancer, cardiovascular diseases, and hypertension) to saturated fatty acid consumption. Hence, at present, the health-conscious consumers request food products with a low content in saturated fatty acids and rich in polyunsaturated fatty acids (PUFA) because they perceive the first ones like a *hazard* for their health status.

This has strongly propelled research with the aim to modify the profile of milk fat, or to reduce its content, to satisfy the consumer’s demand for long chain unsaturated fatty acids, especially oleic acid C18:1, linoleic acid C18:2 n6, and linolenic acid C18:3 n3 because of their potential anticarcinogenic and cardioprotective roles. (La Vecchia, 2009; Lavie et al. 2009)

Nutrition is the major environmental factor affecting bovine milk fat (Chilliard et al 2004; Bourre 2005) and represents a practical tool to alter its composition. It is generally recognized that the fatty acid content of a diet consumed by lactating cows affects both the type and the relative proportion of the fatty acids.

Feeding strategies require the use of supplements to meet nutritional demand and to improve the quality and FA composition of milk fat.

Linseeds resulted suitable feed ingredients, due to their high content of polyunsaturated long chain fatty acids. However bacterial ruminal hydrogenation of dietary unsaturated fatty acids is extensive, thus limiting intestinal flow and subsequent incorporation of diet-derived unsaturated fatty acids into milk fat. Hence, the interest to protect oil seeds from ruminal bio-hydrogenation.

Heat treatment is commonly used to this end.
The application of heat to high fat products, such as linseeds, can denature the protein matrix surrounding the fat droplets with a consequent protection of the fat from ruminal bio-hydrogenation.
Extrusion is a heat treatment process that can be used to protect oils seeds from ruminal degradation.
The objective of this study was to determine the effect of linseeds supplementation, particularly extruded linseeds on incidence of mastitis, on the animal wellness and on milk quality related to fatty acid composition.

Material and methods
The study on the effect of feedstuff supplementation with linseeds on milk tocopherol and unsaturated fatty acid content was conducted in an experimental dairy herd at CRA-PCM (CRA-PCM: Centro di Ricerca Agroalimentare per la Produzione delle Carni e il Miglioramento genetico Roma-Tor Mancina) in the central part of Italy. Twenty dairy cows (Frisona Italiana), starting from 30 days after delivery, were selected on the basis of: number of lactations, lactation period and BSC, fertility records, stillborn calves and monthly milk production. The selected animals were divided into 2 homogeneous groups (experimental: LE and control: C) and studied for a period of 11 months. Animals under study were housed in two different enclosures in a separate area of the farm.
Both groups received a control diet correspondent to a mean quantity of 17.55 kg dry matter/animal/day during the whole experimental period. In addition to the control diet, group LE received a daily supplementation of extruded linseeds of 1 Kg /animal/day. This quantity, equivalent to 0.98 UFL, was chosen to make this supplementation irrelevant on the total daily energetic intake meaning 12 UFL/animal group C/day vs LE 12.98 (12+0.98) UFL/ animal group LE/day. None of the animals had access to the pasture during the experiment. Sample collection for the study was carried out monthly. On each collection day, at the afternoon milking (about 4.00 p.m.) and before the normal milking of each animal, 4 sterile samples of about 5 ml from each udder and an additional sample of the whole milk of the same animal, were collected. All the samples were immediately shipped in refrigerated conditions to the laboratory and kept deep-frozen (-20°C) until the analysis. In total, 700 samples collected (336 samples from group C and 364 from group LE). Each sample was tested for bacteriological count on Blood agar and Edward Medium Modified. The research and the identification of the major mastitis pathogens (Corynebacterium *bovis*, Staphylococcus *aureus*, *Serratia marcescens*, *Streptococcus agalactiae*, *Escherichia coli*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*) were performed through biochemical and molecular methods. Milk yield, milk conductivity and rate of flow were evaluated by *Lactocorder*. In total 175 flow curves were registered and the total time of milking was calculated as the sum of the three phases of the flux (increase, plateau, decrease). Chemical–physical analyses (175 individual samples) were performed by Milkoscan FT 6000 and Fossomatic 5000 for the determination of: fat, proteins, lactose, skimmed dry matter, cryoscopy, urea, caseins, somatic cells and PUFA as suggested by Hedegaard et al ,2006. Finally, tocopherol was tested in 220 samples (90 samples for group LE plus 90 samples for group C) by HPLC-UV.

Diets

The 2 groups of cows were fed respectively with: Unifeed (group C) and Unifeed (same quantity of group C) plus extruded linseeds (group LE) in the amount of 1 Kg/animal/day). Later on are reported the tables with the related chemical determinations.

Composition and nutritional value of Unifeed and of extrused Linseed are reported respectively in Tables 1, 2 and 3.

Table 1. Composition of Unifeed

Composition	% Unifeed composition	% DM composition
hay	16.67	23.97
maize silage	58.97	43.09
maize flour	11.54	14.90
barley grains	3.42	4.89
soy flour	8.97	12.50
alpha	0.43	0.64
Tot	100.00	100.00

Table 2. Nutritional composition of Unifeed (on dry matter)

	%
dry matter 100°C	96.53
ash	4.64
proteins	19.68
EE ethereal extract	2.28
FG fibre	11.32
NDF hemicellulose cellulose lignin	36.32
ADF cellulose lignin	15.46
ADL lignin	2.09
UFL	0.92

Table 3. Nutritional composition of extruded linseeds

NDF	26.1
ADF	18.3
ADL	5.5
UFL	0.98

The fat content and natural tocopherol content are reported in Table 4 and Table 5 respectively

Table 4. Fat content of Unifeed and extruded linseeds

Sample	**fat%**
Unifeed	1.71
extrused linseeds	22.4

Table 5. α-tocopherol content of Unifeed and extruded linseed (*values obtained by 3 replicates*)

	Unifeed	extruded linseed
α -tocopherol mg/kg	43±0.9	6.5±0.4
U.I./kg	64	9.6

Statistical analyses

Statistical analysis was performed with GLM (SAS), to evaluate the effect of the treatment and of the lactation period and the combination of treatment x length of time treatment on the parameters considered (milk production, flow curves, and physicochemical parameters.

RESULTS

All the cows with a recovery of mastitis pathogens in the milk sample of at least one quarter were recorded as affected by mastitis (positive). Results are reported in table 6.

A total of 175 (= 100%) individual udder samples (consisting of 4 sub-samples for the 4 quarters) was analysed for the presence of bacterial mastitis pathogens (*Corynebacterium bovis, Staphylococcus aureus, Serratia marcescens, Streptococcus agalactiae, Escherichia coli, Streptococcus uberis,* and *Streptococcus dysgalactiae*).

Fifty-four udders (=30.9%) resulted positive at this research for the presence of pathogens in the milk of at least one quarter. One hundred and twenty one udders (69.1%) resulted negative. The differences between group LE and group C (33,0% vs 28,6%) were statistically significant (P<0,05).

Table 6. Percentage of contaminated quarters by mastitis pathogens (positives) vs negative ones (figures related to the entire period of experiment)

	Group C (n=84)	Group LE (n=91)	Total (n=175)
Positives	24 (28.6%)	30 (33.0%)	54 (30.9%)
Negatives	60 (71.4%)	61 (67.0%)	121 (69.1%)
Total	84 (100.0%)	91 (100.0%)	175 (100.0%)

The same results are expanded in function of the length of the experiment and of the number of infected quarters/cow in Tables 7 and 8 for C and LE groups, respectively.

For group C, in the whole period of observation, 287 quarters resulted negative to the bacteriological research with respect to 49 positive samples (this last number is referred to 24 cows). For the group LE, in the same period of experiment, 310 quarters resulted negative to the bacteriological research respect to 54 positive samples (this last number is referred to 30 cows).

Table 7. Group C: prevalence of infected animals in the time (with details on the n° of infected quarters)

	Month of experiment											**total no infected quarters**
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		
cows distribution with no infected quarters (0/4)	40	36	37	32	34	30	30	25	21	2	287	
cows distribution according to the n° of infected quarters											total infected cows	
1/4		1		2		1	1	1	1		7	7
2/4					1	1	1	3	2	1	9	18
3/4		1	1	2		1	1		2		8	24
4/4											0	0
total infected cows	0	2	1	4	1	3	3	4	5	1	24	49
animal positives to individual controls	0	2	1	4	1	3	3	4	5	1	**24**	

Table 8. Group EL: prevalence of infected animals in the time (with details on the n° of infected quarters)

	Month of experiment											total no infected quarters
	0	1	2	3	4	5	6	7	8	9		
cows distribution with no infected quarters 0/4	40	34	36	38	39	29	23	29	27	15		310
cows distribution according to the number of infected quarters											total infected cows	total N° infected quarters
1/4		3	2	2	1	2	2	2	1		15	15
2/4			1			3	2	1		1	8	16
3/4		1				1	1	1		1	5	15
4/4									1	1	2	8
total infected cows	0	4	3	2	1	6	5	4	2	3	30	54
animal positives to individual controls	0	4	3	2	1	6	5	4	2	3	**30**	

The number of infected animals/ month distributed according to correspondent mastitogenic agents isolated in the milk are reported in table 9. The results indicate a slightly higher number of infected animals in group EL vs C. Results overlaps in the first four months of the experiment, whereas in the second period, the group EL seems to be more sensitive to bacterial infections probably due to the increased milk production.

Table 9. Number of infected animals (n) in the time and distribution according to the causative mastitis agent

	T1	T2	T3	T4	T 5	T 6	T 7	T 8	T 9	Total infected cows/mastitis agent
group C	**n**	**n**	**n**	**n**	**n**	**n**	**n**	**n**	**n**	**n**
Corynebacterium bovis	1		1		1	2	3	1		**9**
Escherichia coli	1			1						**2**
Serratia marcescens		1	1					1		**3**
Streptococcus uberis										**0**
Staphylococcus aureus			1		1	2				**4**
Streptococcus agalactiae					1		1	1	1	**4**
S.aureus+ S. agalactiae								1		**1**
E. coli + S.marcescens								1		**1**
total										**24**
group EL										
Corynebacterium bovis	1	1	2			3	1	1		**9**
Escherichia coli	1	1		1						**3**
Serratia marcescens	2	1				1				**4**
Streptococcus uberis					2					**2**
Staphylococcus aureus					4				2	**6**
Streptococcus agalactiae							2			**2**
S. aureus+ S.marcescens								1		**1**
C.bovis+ S. agalactiae						1				**1**
S. agalactiae+E. coli							1			**1**
S. aureus+S.agalactiae									1	**1**
total										**30**

None of the animals included in the group was infected at T0

Results for milk yield and the details of the major parameters of the flow curves are reported in tables 10 and 11.

Table 10. Milk yield and details of the major parameters of the flow curves in the 2 groups

Parameters	Group C	Group LE
Milk Yield (Kg/milking)	9.45±0.38 [a]	11.21±0.38 [b]
Maximum flow (Kg/min)	3.29±0.08	2.80±0.09
Average Flow (min)	2.10±0.07	1.87±0.06
Increasing time (min)	1.12±0.05	1.03±0.06
Plateau time (min)	1.09±0.10 [a]	2.38±0.17 [b]
Decreasing time (min)	2.52±0.18	2.66±0.14
Total milking time (min)	4.73±0.19 [a]	6.07±0.23 [b]

[a, b] Different superscript letters, in the same row, indicate the significance level $P<0.05$.

Milk production was higher in the group LE than in the group C (11.21±0.38 kg vs 9.45±0.38 kg; $P<0.001$). Actually group LE received, with respect to the control group, a supplement of 1 kg of extruded linseed/animal/day, equivalent to 0.98 UFL. This accounts for the apparent different result obtained by other authors who described lower milk productions in animals fed with extruded linseeds when both the animal groups (control and test) were given diets with equal energy content.

Plateau duration was longer for LE group than for C group (2.38±0.17 min vs 1.09±0.10; $P<0.001$). This was related to different reasons, basically a higher milk yield in group LE, and by a more uniform distribution of the milk in the quarters (fore quarters vs back quarters). The longer plateau time resulted also in a longer total milking time in group LE respect to group C (6.07±0.23 min vs 4.73±0.19 min; $P<0.01$). The same parameter, recorded at time 0 (before the first supplementation of extruded linseeds) had proven a no statistically significant difference between the 2 groups of cows.

Table 11. Milk yield in the course of the experiment

Milk production (kg)	Group C	Group LE
T 0	12.77±0.97	14.53±0.92
T 1	11.63±0.97	13.51±0.92
T 2	10.02±0.92	10.62±0.92
T 3	10.03±0,92	12.58±0.92
T 4	9.52±0.97	12.78±0.92
T 5	7,.5±0.97	10.33±0,92
T 6	8.34±1.03	10.28±0.97
T 7	7.38±1.10	7.79±0.97
T 8	8.15±1.10	8.44±1.10
T 9	6.26±1.19	7.24±1.19

Results of chemical and physical parameters are reported in Table 12.

Table 12. Chemical and physical parameters on milk tested

	Group C	Group LE
Milk Composition (%)		
fat	3.92±0.12 a	3.41±0.11 b
protein	3.45±0.04 a	3.37±0.04 b
lactose	4.74±0.02 a	4.87±0.03 b
defatted dry matter	8.88±0.04 a	8.94±0.04 b
cryoscopy °C	- 0.532±0.002	- 0.532±0.002
urea mg/dl	23.9±0.6 a	22.6±0.6 b
caseine	2.56±0.03 a	2.55±0.03 b
SCC * 1.000	742±212	546±103
Milk Components(g/milking)		
fat	345.7±11.0 a	372.9±15.8 b
proteins	311.7±13.5 a	369.6±11.7 b
lactose	439.8±18.1 a	542.5±18.8 b

a, b Different letter superscript indicate the significance level P<0.05.

Milk composition and milk component yield were lightly influenced by the linseed supplementation. This variation was observed in all the cows, although in different degree.

As reported in Table 13, the fat percentage was lower in group LE than in group C (3.41±0.11% vs 3.92±0, 12%; P<0.01). This result is in agreement with previous observations of other authors who noticed that a supplement of fat in the animal diet can result in a lower fat milk content. This is probably due to a feedback mechanism started by intermediate products synthesised in the rumen that cause the stop of *ex novo* fat synthesis in milk (Morales et al, 2000; Loor and Herbein, 2003).

Protein production (Table 14) was higher in group C than in group LE (3.45±0.04% vs 3.37±0.04%; P<0.001). This result too is consistent with the observation reported in the literature. Gonthier et al., (2004) reported that a fat supplement in the diet results in a lower protein content in the milk. Different hypotheses might be involved in this observation. Gonthier et al., (2004) went back to a reduced protein synthesis due to a lower availability of aminoacids consequent to lower duodenal flux of microbial proteins.
The altered metabolism of insulin or glucose seems also responsible of the reduction of the percentage of protein in the milk (Dhiman et al, 1995).

Table 13. Fat content (%) during the experiment

Fat (%) in milk	Group C	Group LE
T 0	3.18±0.34	3.31±0.32
T 1	3.66±0.34	3.04±0.32
T 2	4.12±0.32	3.22±0.32
T 3	3.41±0.32	2.82±0.32
T 4	3.85±0.34	3.20±0.32
T 5	3.95±0.34	3.20±0.32
T 6	4.06±0.36	4.18±0.34
T 7	4.20±0.38	3.84±0.34
T 8	3.99±0.38	3.39±0.38
T 9	4.82±0.41	3.92±0.41

Table 14. Protein content (%) during the experiment

Proteins (%) in milk	Group C	Group LE
T 0	2.99±0.10	3.01±0.10
T 1	3.22±0.10	3.16±0.10
T 2	3.38±0.10	3.21±0.10
T 3	3.25±0.10	3.13±0.10
T 4	3.39±0.10	3.40±0.10
T 5	3.68±0.10	3.38±0.10
T 6	3.43±0.11	3.58±0.10
T 7	3.77±0.11	3.57±0.10
T 8	3.65±0.11	3.89±0.11
T 9	3.76±0.12	3.63±0.12

Surprisingly lactose percentage (Table 15) was slightly higher in group LE with respect to group C (4.87±0.03 % vs 4.74±0.02 % but statistically significant ($P<0.05$).

Table 15. Lactose content (%) during the experiment

Lactose (%) in milk	Group C	Group LE
T 0	4.86±0.07	4.85±0.07
T 1	4.74±0.07	5.00±0.07
T 2	4.78±0.07	4.92±0.07
T 3	4.76±0.07	4.91±0.07
T 4	4.76±0.07	4.99±0.07
T 5	4.78±0.07	4.87±0.07
T 6	4.71±0.08	4.71±0.07
T 7	4.64±0.08	4.78±0.07
T 8	4.72±0.08	4.75±0.08
T 9	4.72±0.09	4.84±0.09

Somatic cell count (SCC) (table 16) seemed to be higher in group C than in group LE (742±212 cell/ml vs 546±103 cell/ml) but no significant differences were observed.

Table 16. SCC content (cell x 1000/ml) during the experiment

SCC	Group C	Group LE
T 0	237±304	901±280
T 1	677±304	124±288
T 2	445±288	556±288
T 3	497±288	632±288
T 4	408±304	104±288
T 5	661±304	743±288
T 6	518±322	700±304
T 7	983±344	365±304
T 8	699±345	744±345
T 9	410±372	670±372

Table 17. casein content (%) during the experiment

Casein (%) in milk	Group C	Group LE
T 0	2.77±0.07	2.29±0.07
T 1	2.46±0.07	2.47±0.07
T 2	2.57±0.07	2.44±0.07
T 3	2.43±0.07	2.38±0.07
T 4	2.57±0.07	2.61±0.07
T 5	2.74±0.07	2.55±0.07
T 6	2.54±0.08	2.64±0.07
T 7	2.75±0.08	2.67±0.07
T 8	2.71±0.08	2.90±0.08
T 9	2.76±0.09	2.68±0.09

Tocopherol content in milk was analysed according to Hedegaard RV et al (2006) and values, reported in Table 18, are expressed as mean of 10 samples (+ S.D.)
Alfa–tochopherol content in milk did not decrease in group LE unlike other milk components (see Table 12). This seems to be of little influence for milk quality and for its derivates. Havemose et al. (2004) found that α-tocopherol only had an effect on delaying the dityrosine formation but no effect on the lipid oxidation. In addition, the supplementation of vitamin E in the diet of dairy cows did not seem to prevent unsaturated fatty acid degradation (Sympoura et al 2009).

Table 18. Alfa - tochopherol in milk (mg/100ml)

	Group C	Group LE
T 0	0.06±0,01	0.11±0.02
T 1	0.07±0.01	0.08±0.01
T 2	0.07±0.01	0.10±0.01
T 3	0.08±0.01	0.10±0.02
T 4	0.10±0.02	0.11±0.01
T 5	0.08±0.02	0.07±0.02
T 6	0.07±0.01	0.03±0.01
T 7	0.08±0.03	0.07±0.01
T 8	0.08±0.01	0.06±0.02
T 9	0.10±0.02	0.08±0.02

The body condition scoring (BCS) which is an useful, easy-to-use management tool to determine the nutritional needs of animals is reported in Table 19.

Table 19. BCS profile

BCS	Group C mean/sd	Group LE mean/sd
T 0	2.56±0.32	2.58±0.31
T 1	2.63±0.23	2.69±0.24
T 2	2.66±0.19	2.75±0.31
T 3	2.59±0.27	2.69±0,24
T 4	2.59±0.27	2.72±0,20
T 5	2.66±0.27	2,69±0.24
T 6	2.75±0.23	2.69±0.24
T 7	2.72±0.16	2.75±0.18

The results of milk fatty acid profile in experimental groups of lactating cows and their relative concentrations are reported in Table 20. As reported in literature, extruded flaxseed supplementation can modify the fat profile in milk, particularly increasing the concentration of some unsaturated fatty acids.(Ward et al 2002; Petit 2003).

Table 20. Milk fatty acid profile of lactating cows fed extruded linseed (mg/g of fatty acid)

	Group C	**Group LE**
C14:0	115	75
C16:0	312	206
C16:1	12	9
C18:0	112	170
C18:1 trans	23	53
C18:1 cis	215	309
C18:2	21	28
C18:3	4	9
CLA	8	16
MCFA	473	315
LCFA	380	595
PUFA	31	52
Saturated	703	560

MCFA monounsaturated fatty acid
LCFA long chain fatty acid
PUFA polyunsaturated fatty acid
CLA mainly $C_{18:2\ cis\ 9,trans\ 11}$

CONCLUSIONS

Although feeding extruded linseed at about 6% of dietary dry matter to dairy cows, seems to improve the health status of the animals, as indicated by the observation of several metabolic parameters-- the same diet seems to be effective neither on the prevalence of mastitis nor on the tocopherol content of milk. However, these results are likely to be influenced by the small number of animals used in the experiment.
The linseed supplementation proved to be effective to modulate the fatty acid composition and to obtain appreciable increases in unsaturated fatty acids.
The lack of significant difference in tocopherol content should be taken into account since the success of a richer content in PUFA milk needs a richer content of antioxidant to avoid degradation and sensory modifications.
As a further consideration, prolonged higher excretion of milk could have a detrimental effect on the animal welfare if not well balanced and supported. So, considering the importance of vit E in keeping cow's health status, on the basis of our results, it seems advisable, when higher milk yield are obtained, to integrate the ratio of dairy cows of tocopherol with a double finality: as antioxidant (for the milk) and integrator (for the animal).

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