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FINAL REPORT

The effect of protected polyunsaturated fatty acids in dairy cows on fertility, production performance and milk fatty acid profiles

DAIRY HERD DOBROSEV A.S., CZECH REPUBLIC, 2012

Summary

The aim of the study was to evaluate the effect of dietary supplementation of dairy cows with different levels of feed additive containing protected polyunstaturated fatty acids (SALMATE®) on fertility, performance and fatty acid profiles in milk fat. The study was performed under field conditions in a herd of 500 cows with average yearly milk yield of 11,000 kg. The cows were given total mixed ration based on maize silage, clover-grass silage, hey, straw, concentrate and mineral-vitamin supplement. The tested product was mixed with wheat flour and administerd as top-dressing on morning meals.

The trial included 99 cows divided into 3 groups:

1) Control (K): total mixed ration without SALMATE (n=33)
2) OMEGA 40 (O40): total mixed ration + SALMATE at 40g/cow/day from 30 to 90 days in milk (n=33)
3) OMEGA 50 (O50): total mixed ration + SALMATE at 50g/cow/day from 50 to 90 days in milk (n=33)

Performance parameters (milk yield, milk fat, milk protein, somatic cell counts) were monitored. Fatty acid profiles were determined in pooled milk samples, each from 10 cows.
The following fertility parameters were recorded: calving to first oestrus interval, calving to first service interval, calving to conception interval and number of services per conception (insemination index).
In 8 cows from each group progesteron levels in blood were determined on the day of AI service, 12-15 days post AI and 20 days post AI, and probability of embryonal mortality was assessed. For the same 8 cows metabolic profile was determined (total protein, albumin, urea, AST, billirubin, cholesterol, glucose, BHB, NEFA, P, Ca, Zn, Cu, betacarotene, T3, T4).

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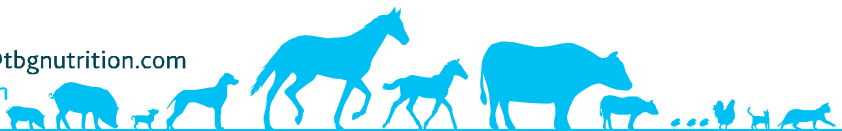

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Results:
Average milk yield till 100 DIM was significantly higher in the O50 group than in the Control, in first parity heifers there was statistical significance ($P < 0,05$). Estimated total milk fat per 100 DIM was significantly increased by both the SALMATE treatments as compared with the Control. Somatic cell counts were not influenced.
There were no significant differences in average calving to conception intervals till 150 DIM between the groups (K – 97d, O40 – 96d, O50 – 84d). Insemination index (no. of services per conception) in cows pregnant before 150 DIM was the lowest in the group O40 (1.45), then in O50 (1.56) and the highest in the Control (1.67).

Total numbers of cows that conceived before 120 and 150 DIM were the highest in the O40 group (20 and 19, respectively), then in O50 (18 and 15), and the lowest in the Control (17 and 13).

The highest number of cows suspected of early embryonic mortality was in the Control group (K- 13 cows, O40 – 9 cows, O50 – 10 cows). As indicators of suspected embryonic mortality progesteron dynamics (drop in progesterone levels) in blood, delayed subsequent oestrus and negative result of ultrasound pregnancy detection 30 days post service were taken.
Mean values of metabolic parameters were very similar between the groups and no significant differences were found. The values were within the physiological range and indicated a well-balanced energy status of the cows.

Fatty acid profiles of milk fat were not markedly influenced by the experimental supplementation, only alpha-linolenic acid (C18:3n3) was slightly decreased in milk from cows supplemented with SALMATE.

2. Introduction and aim of study

A supplementation of dairy cows with long chain polyunsaturated fatty acids (PUFA) is a recent trend in dairy cow nutrition. Many studies have shown favourable effects of omega-3 polyunsaturated fatty acids, protected from ruminal biohydrogenation, on reproductive performance in dairy cows. PUFA have been reported to contribute to reduction in synthesis of proinflammatory prostaglendin $PGF_{2\alpha}$ in the endometrium, probably due to a reduced synthesis of arachidonic acid which is a precursor of $PGF_{2\alpha}$, in favour of competitve synthesis of eicosapentaenoic acid (Mattos, 2010). Essential fatty acids are also important for progesterone synthesis. Omega 3 (n-3) polyunsaturated fatty acids have been reported to improve fertility in dairy cows (Petit et al., 2001).

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Several studies have reported that protected PUFA if provided in higher amounts, may alter fatty acid milk fat composition by increasing levels of n-3 polyunsaturated fatty acids. Fish oil is a rich source of n-3 fatty acids (EPA, DHA).
SALMATE contains approximately 45% of fish oil encapsulated with starch matrix, providing polyunsaturated fatty acids – eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6).

The aim of this study was to ivestigate the effects of supplementation with protected polyunsaturated fatty acids at different levels and durations on reproductive parameters, production performance and fatty acid profiles in milk fat of dairy cows.

3. Trial site

Dobrosev, a. s.
Dobronín 188
58812 Dobronín
Czech Republic

500 cows, average milk yield 11 000 kg. Loose housing, 40-50 cows in one pen.

4. Duration of experiment

Beginning: June 2012
End: December 2012

5. Material and methods

5.1 Animals

Status:	lactating dairy cows
Breed:	Holstein
Randomization:	Cows were randomized according to parity, calving date and previous lactation yield.

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5.2. Feeding and administration of the product tested

The experimental cows were fed total mixed ration (TMR) consisting of maize silage, clover-grass silage, hey, straw and mineral concentrate.

SALMATE was mixed with wheat flour at the levels of 0g SALMATE/100g blend (0%) (K), 40g SALMATE/100 g blend (40%) (O40) and 50g SALMATE/100g mix (50%) (O50). The experimental blends were given on top of TMR at morning meals, 100g of blend per cow.

5.3 Experimental treatments

4) Control (K): TMR + 0% blend
5) OMEGA 40 (O40): TMR + 40% blend from 30 to 90 DIM
6) OMEGA 50 (O50): TMR + 50% blend from 50 to 90 DIM

Numbers of cows per treatment

Treatment	N
Control	33
OMEGA 40	33
OMEGA 50	33

5.4 Parameters under study

Milk yield, milk fat, milk protein, somatic cell counts
The milk testing paramters were recorded monthly.

Fatty acid profiles in milk fat

- Samples of milk were taken on 30(50), 75 and 90 DIM. For each treatment a pooled sample from 10 cows (the same cows each time) was taken. There were 9 pooled samples in total.
- Milk acid profiles were analyzed at the Institute of Chemical Technology Prague, Faculty of Food and Biochemical Technology, Prof. Jana Hajšlová.

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Metabolic profile

- Total protein, albumine, urea, AST, billirubin, choleterol, glucose, BHB, NEFA, P, Ca, Zn, Cu, betacarotene, T3, T4
- 8 cows per treatment
- 3 times (at AI, 12-15 days post AI, 20 days post AI)

Activity

Activity of cows was measured by pedometer and recorded in the milking parlour software. Based on activity, the interval from calving to first heat was determined.

Reproduction parameters

- Calving to first AI interval
- Calving to conception interval
- Insemination index (number of services per one conception)
- Ultrsound pregnancy testing results

Serum progesterone levels

- Corpus luteum function, detection of embryonic mortality
- 8 per treatment
- 3 times (at AI, 12-15 days post AI, 20 days post AI)

5.5 Statistical evaluation

The raw data were processed in Microsoft Excell. Mean values (X), standard deviations (S.D.) were calculated. The statistical significance of differences between median values was tested by non-paired parametric Sutent´s t-test. The difference was regarded as statistically significant from $P < 0,05$.

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6. Results

6.1 Cows

Table 1: Average parity

Treatment		Current parity
CONTROL	**MEAN**	**1,91**
	S.D.	1,08
OMEGA 40	**MEAN**	**1,88**
	S.D.	1,22
OMEGA 50	**MEAN**	**2,09**
	S.D.	1,00
Hladina významnosti		
P	K x O40	NS*
P	K x O50	NS
P	O40 x O50	NS

* NS…non-significant difference

6.2 Milk performance results

Mean milk yield till 100 DIM in the current lactation (experimental) was the highest in the O50 ($P < 0.05$) in first parity heifers as well as in cows (parity 2+). If previous parity 100 DIM milk yield is taken as covariate, an increase is the greatest in the O40 group (non-significant). Total kg of milk fat till 100 DIM calculated with mean fat percentage measured till 120 DIM is significantly highest in the O50 group. There were not significant differences in milk protein between the treatments. Mean somatic cell counts were not significantly different, however, in groups O40 and O50 thy were numerically lower than in the Control (Table 4).

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Table 2 Average milk yield till 100 DIM

		All parities	All parities	Parity 2+	Parity 1	Parity 2+
		Milk yield	Milk yield	Difference	Milk yield	Milk yield
		100 DIM	100 DIM	100 DIM	100 DIM	100 DIM
		previous	current	current-previous	current	current
		kg	kg	kg	kg	kg
CONTROL	MEAN	**2798**	**3076**	**582**	**2842**	**3351**
	S.D.	788	713	849	531	771
OMEGA 40	MEAN	**2786**	**3215**	**678**	**2908**	**3495**
	S.D.	412	578	765	445	569
OMEGA 50	MEAN	**3090**	**3514**	**573**	**3251**	**3664**
	S.D.	511	714	818	381	810
K x O40	*P*	NS	NS	NS	NS	NS
K x O50	*P*	NS	< 0.05	NS	< 0.05	NS
O40 x O50	*P*	< 0.1	< 0.1	NS	< 0.05	NS

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Table 3 Mean milk fat and protein percentages in the current lactation

		30 DIM	30 DIM	60 DIM	60 DIM	90 DIM	90 DIM	120 DIM	120 DIM	150 DIM	150 DIM
		Fat	Protein	Fat	Protein	Fat	Protein	Fat	Protein	Fat	Protein
		%	%	%	%	%	%	%	%	%	%
CONTROL	**MEAN**	**4,16**	**3,22**	**3,88**	**3,20**	**3,84**	**3,29**	**4,07**	**3,46**	**4,29**	**3,60**
	S.D.	0,70	0,29	0,77	0,32	0,78	0,27	0,84	0,24	0,83	0,28
OMEGA 40	**MEAN**	**4,16**	**3,14**	**3,92**	**3,18**	**3,83**	**3,29**	**3,96**	**3,46**	**4,20**	**3,59**
	S.D.	0,60	0,29	0,62	0,19	0,75	0,18	0,63	0,22	0,68	0,21
OMEGA 50	**MEAN**	**4,20**	**2,96**	**3,99**	**3,23**	**4,02**	**3,18**	**3,70**	**3,48**	**4,18**	**3,61**
	S.D.	0,91	0,21	0,80	0,29	0,78	0,26	0,60	0,28	0,72	3,45
P	K x O40	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P	K x O50	NS	< 0,001	NS	NS	NS	NS	< 0,1	NS	NS	NS
P	O40 x O50	NS	< 0,05	NS	NS	NS	< 0,1	NS	NS	NS	NS

Table 3a Total milk fat and milk protein per 100 DIM

	Total fat in 100 DIM	Total protein in 100 DIM
	kg	**kg**
CONTROL	**121,3**	**98,9**
	29,4	20,9
OMEGA 40	**126,3**	**98,7**
	26,1	17,0
OMEGA 50	**146,1**	**108,1**
	41,6	23,8
K x O40	NS	NS
K x O50	< 0,05	NS
O40 x O50	< 0,01	< 0,1

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Table 4 Mean somatic cell counts

		30 DIM	60 DIM	90 DIM	120 DIM	150 DIM
		PSB (SCC)	PSB	PSB	PSB	PSB
		Thous.	Thous.	Thous.	Thous.	Thous.
CONTROL	**MEAN**	**295**	**518**	**220**	**109**	**96**
	S.D.	685	1771	637	178	118
OMEGA 40	**MEAN**	**377**	**221**	**153**	**902**	**280**
	S.D.	1168	532	191	2121	500
OMEGA 50	**MEAN**	**73**	**188**	**88**	**191**	**108**
	S.D.	169	322	101	502	70
P	K x O40	NS	NS	NS	NS	NS
P	K x O50	NS	NS	NS	NS	NS
P	O40 x O50	NS	NS	NS	NS	NS

6.4 Reproduction parameters

The reproduction results are given in Table 5. Mean calving to first oestrus and calving to first service intervals were the shortest in the Control group, the differences were not significant. Mean calving to conception intervals till 150 DIM were 97d for Control, 96d for O40 adn 84d for O50, the differences were not significant. Mean insemination index for conceptions before 150 DIM was 1.45, 1.56 and 1.67 for O40, O50 and Control, respectively. However, the differences were not significant.

Total number of pregnant cows before 120 DIM and 150 DIM was the highest in O40 (20 and 19, respectively), than in O50 (18 and 15, respectively), than in Control (17 and 13, respectively.

In the Control group the highest number of cows were suspected of early embryonic death (K-13, O40 – 9, O50 - 10). The assumptions were based on progesteron levels, delayed subsequent osestrus, ultrasound pregnancy detection.

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Table 5 Fertility results

		Calving to 1st oestrus	Calving to 1st AI	Calving to conception before 150 DIM	No.of AI per conception before 150 DIM		No.of cows conceiving before 150/120 DIM	Suspected embryonic mortality(persisting corpus luteum
CONTROL	**MEAN**	**54,35**	**78,74**	**96,89**	**1,67**	**Σ**	**17/13**	**13**
	S.D.	33,54	22,66	25,77	0,67			
OMEGA 40	**MEAN**	**72,30**	**86,36**	**95,77**	**1,45**	**Σ**	**20/19**	**9**
	S.D.	35,86	27,61	18,56	0,72			
OMEGA 50	**MEAN**	**60,04**	**85,95**	**83,50**	**1,56**	**Σ**	**18/15**	**10**
	S.D.	30,42	32,09	28,52	0,83			
P	K x O40	< 0,1	NS	NS	NS			
P	K x O50	NS	NS	NS	NS			
P	O40 x O50	NS	NS	NS	NS			

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Tables 6-8 Serum progesterone levels post service

OMEGA 50				
Cow	DIM at service	Conception 1 = yes	Suspected embryonic mortality	Progesterone (ng/ml)
393792	71	1		0,37
				4,87
				4,06
393698	51	1		0,18
				4,33
				4,63
363202	94	1		0,38
				2,11
				2,31
393607	63	0		0,27
				0,39
				0,33
393567	61	1		0,34
				4,13
				5,71
393767	70	0	1	0,31
				2,36
				1,88
326673	97	0		0,31
				0,33
				0,42
393720	131	0	1	0,34
				0,97

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OMEGA 40				
Cow	DIM at service	Conception 1 = yes	Suspected embryonic mortality	Progesterone (ng/ml)
393730	47	0	1	0,22
393730				1,86
393730				1,34
363169	84	0	1	0,22
363169				1,37
363169				0,66
363179	100	1		0,34
363179				5,32
363179				5,71
326679	65	0	1	0,37
326679				1,63
326679				0,88
393831	56	0	1	0,22
393831				1,75
393831				1,67
393831				1,63
393749	109	1		0,41
393749				3,51
393749				5,01
326705	41	0	1	0,47
326705				2,86
326705				3,37
326855	84	1		0,23
326855				2,36
326855				4,33

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Tabulky 6-8 Vyšetření hladin progesteronu po inseminaci

CONTROL				
Cow	DIM at service	Conception 1 = yes	Suspected embryonic mortality	Progesterone (ng/ml)
363159	86	0	1	0,19
363159				1,82
363159				0,98
393789	115	0	1	0,31
393789				1,14
393789				2,41
393815	58	1		0,19
393815				3,86
393815				4,27
393732	86	1		0,37
393732				2,36
393732				4,88
393842	66	0	1	0,23
393842				1,94
393842				0,31
363216	47	0	1	0,19
363216				1,33
363216				0,18
393838	50	0	1	0,37
393838				1,38
393838				0,34
393837	101	1		0,31
393837				5,02
393837				4,72

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6.5 Metabolic profile

Mean values of all the metabolic parameters measure were similar between the treatments and no significant differences were found (Table 9). The values were within physiological ranges and indicated a well-balanced energy status.

Table 9 Mean values of metabolic parameters

		Cb	**Alb**	**Gluk**	**Urea**	**NEFA**	**BHB**	**Chol**	**AST**
		g/l	g/l	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	ukat/l
O50	**MEAN**	**73,77**	**33,33**	**3,06**	**5,58**	**0,56**	**0,70**	**4,38**	**1,51**
	S.D.	2,84	2,10	0,18	0,48	0,24	0,20	0,53	0,18
O40	**MEAN**	**73,45**	**33,10**	**3,07**	**5,47**	**0,55**	**0,68**	**4,52**	**1,47**
	S.D.	2,75	1,87	0,18	0,56	0,15	0,29	0,55	0,14
K	**MEAN**	**73,03**	**33,02**	**3,09**	**5,55**	**0,50**	**0,72**	**4,30**	**1,48**
	S.D.	2,98	1,45	0,20	0,50	0,13	0,23	0,54	0,16
T-test	**O50xO40**	NS	NS	NS	NS	NS	NS	NS	NS
	O50xK	NS	NS	NS	NS	NS	NS	NS	NS
	O40xK	NS	NS	NS	NS	NS	NS	NS	NS

		Ca	**P**	**Zn**	**Cu**	**betakar.**	**T4**	**T3**
		mmol/l	mmol/l	umol/l	umol/l	umol/l	nmol/l	nmol/l
O50	**PRŮM**	**2,34**	**1,95**	**13,22**	**12,24**	**6,07**	**50,56**	**1,71**
	S.D.	0,05	0,10	1,08	1,02	0,63	14,00	0,12
O40	**PRŮM.**	**2,35**	**1,96**	**12,97**	**11,82**	**5,92**	**53,45**	**1,69**
	S.D.	0,05	0,12	1,57	1,22	0,45	14,64	0,14
K	**PRŮM.**	**2,35**	**1,95**	**13,33**	**12,44**	**5,95**	**51,88**	**1,77**
	S.D.	0,05	0,14	1,30	1,22	0,48	15,88	0,14
T-test	**O50xO40**	NS	NS	NS	NS	NS	NS	NS
	O50xK	NS	NS	NS	NS	NS	NS	NS
	O40xK	NS	NS	NS	NS	NS	NS	< 0,1

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6.6 Fatty acid profiles in milk fat

37 fatty acids were determined in milk fat (Table 10). Alpha-linolenic acid (C18:3n3) levels in O40 and O50 seem to be slightly higher than in the Controls. Levels of cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) and cis-4,7,10,13,16,17-docosahexaenoic acid (C22:6n3) were below their detection limits.

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Milk fatty acid profiles	1st sampling			2nd sampling			3rd sampling		
	K d30	O40 d30	O50 d50	K d75	O40 d75	O50 d75	K d90	O40 d90	O50 d90
Butyric (C4:0)	1,1	1,1	0,8	1	0,4	0,9	1,21	0,9	1,38
Capronic (C6:0)	1,1	1	0,7	1,2	0,3	0,8	0,78	0,8	0,75
Caprylic (C8:0)	0,8	0,6	0,5	0,8		0,6	0,59	0,6	0,51
Caprinic (C10:0)	1,9	1,4	1,4	1,8	0,3	1,3	1,91	1,5	1,23
Undecanoic (C11:0)	0,2	0,1	0,2	0,3		0,1	0,25	0,2	0,13
Lauric (C12:0)	2,5	1,8	2,3	2,5	0,4	1,8	2,89	2,2	1,74
Tridecanoic (C13:0)	0,2	0,1	0,2	0,2		0,1	0,2	0,1	0,1
Myristic (C14:0)	9,4	7,4	9,2	9,9	3,1	7,1	10,8	8,7	7,73
Myristo-oleic (C14:1)	0,6	0,4	1	0,9	0,4	0,4	0,82	0,6	0,52
Pentadecanoic (C15:0)	1,2	1	1,3	1,9	0,4	0,7	1,21	1	0,72
cis-10-pentadecanoic (C15:1)									
Palmitic (C16:0)	30	27,2	32,2	36,1	26,3	28,2	33,48	30,8	29,4
Palmito-oleic (C16:1)	1,5	1,5	2,1	1,6	2,6	1,7	1,52	1,8	1,74
Heptadecanoic (C17:0)	0,7	0,7	0,7	0,8	0,8	0,7	0,67	0,8	0,77
cis-10-heptadecenoic (C17:1)							0,19		0,32
Stearic (C18:0)	8,6	11,2	7,9	6,9	12,1	11,1	8,74	10,2	11,46
Oleic (C18:1n9c)	21,6	23,7	19	17,7	37,8	24,5	20,17	20,8	28,23
Elaidic (C18:1n9t)	2,3	0,2	2,4	1,4	1,1	2	1,9	0,1	1,6
Linoleic (C18:2n6c)	2,9	3	2,7	2,2	2,4	3,4	2,1	2,5	2,15
Linolelaidic (C18:2n6t)	0,2	0,2	0,1	0,2	0,3	0,2		0,1	0,16
alpha-linolenic (C18:3n3)	**0,5**	**0,5**	**0,4**	**0,3**	**0,4**	**0,5**	**0,6**	**0,7**	**0,73**
gamma-linolenic (C183n6)	0,1	0,1	0,1			0,1	0,12	0,2	
arachic (C20:0)	0,1	0,2	0,2	0,1		0,1	0,14	0,2	0,11
cis-11-eicosenic (C20:1n9)	0,1	0,1	0,1	0,2		0,1		0,1	
cis-11,14-eicosadienic (C:20:2)									
cis-11,14,17-eicosatrienic (C20:3n3)									
cis-8,11,14-eicosatrienic (C20:3n6)	0,1	0,1	0,1	0,1		0,1	0,11	0,1	
arachidonic (C20:4n6)	0,1	0,1	0,1	0,2		0,1	0,11	0,1	0,11
cis-5,8,11,14,17-eicosapentaenoic (C20:5n3)								0,1	
heneicosanoic (C21:0)	0,7	0,6	0,8	0,5	0,3	0,6	0,57	0,6	0,49
behenic (C22:1n9)									
erucic (C22:1n9)									
cis-13,16-docosadienoic (C22:2)									
cis-4,7,10,13,16,17-docosahexaenoic (C22:6n3)									

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