

Influence of two flaxseed varieties, differing in fatty acid profile, in dairy cow diets on selected blood indices and reproduction^{*)}

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Summary

The aim of the investigation was to determine the influence of flaxseed of two varieties, differing in their fat composition, on blood indices of cows in the transition period and on their reproductive parameters. Fifty-six multiparous Polish Holstein-Friesian cows were allocated into 3 groups: one control and two experimental (H-ALA and L-ALA). Cows of all groups were fed the same basal diet given in a mix form, and the diets of the experimental groups were enriched with an additive of flaxseed (mixed with concentrate), of either a traditional variety (Szafir, rich in α -linolenic acid, H-ALA group) or a modified variety (Amon, poor in α -linolenic acid but rich in linoleic acid, L-ALA group) given in the amounts of 3% and 6% of diet dry matter (DM) during 6 wks prior to the calving and during 17 wks of lactation, respectively. Blood plasma samples, collected 4 times, were analyzed for the contents of glucose (GLU), urea, total protein (TP), non-esterified fatty acids (NEFA), β -hydroxybutyric acid (BHBA), total cholesterol (CHOL), triacylglycerols (TG), HDL cholesterol fraction, and plasma fatty acid (FA) profile. Reproductive parameters of cows were determined as well. Flaxseed of both varieties did not have any significant influence on the GLU or TG contents in blood plasma. Both the BHBA and NEFA contents in all treatments were much below the undesirable levels and flaxseed supplement decreased additionally ($p \leq 0.05$) the NEFA concentration in plasma in the 2nd wk after calving, with the stronger impact of the traditional flax (decrease by 37% compared with the control cows), which may point to lower adipose tissue mobilization in these animals. The flaxseed additive increased ($p \leq 0.05$) the plasma CHOL content, especially in the case of the modified flax variety. The traditional variety of flax increased ($p \leq 0.05$) the blood plasma level of α -linolenic acid (ALA), which is recognized as a component inhibiting PGF₂ α secretion, thus decreasing embryonic mortality in the first weeks after insemination. However, we did not note any significant improvement in the reproductive indices of cows fed seeds of this flax variety. The results suggest that diets enriched with flaxseed of the traditional variety (rich in ALA) in the amount of 6% of diet DM in early lactation increase the blood plasma level of this FA without causing a considerable improvement in cows' reproductive parameters.

Keywords: flaxseed variety, fatty acids, cows, blood indices, reproductive parameters

At the start of lactation of high yielding dairy cows, a negative energy balance due to the low feed intake is noted in their organism. The insufficient coverage of the glucose requirements by the diet carbohydrates leads to the mobilization of adipose fat with the result that the values of some blood indices may change (6). To compensate for energy deficit, characteristic of early lactation, various feeding strategies have been used, for example, increasing the share of grains (high

percentage of starch escaping ruminal fermentation) and applying glucogenic or fat preparations (14, 16, 18). Fat supplements, which improve energy balance, may influence the milk yield and reproductive indices, as well as modify blood and milk composition. When plant or fish oils are included in the diet, the level of polyunsaturated fatty acids (PUFAs), regarded as essential in diets for monogastric animals, may increase in blood plasma (3). Although most of PUFAs can become biohydrogenated in the rumen, these fatty acids are also important for ruminants, and therefore it may

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be useful to monitor PUFA levels in their blood plasma (15, 21). A very good source of fat for ruminants are oilseeds – sunflower, soybean, and flax. They differ, however, in their fatty acid profiles. The main fatty acid of soybean and sunflower seeds is linoleic acid (LA, C18:2), belonging to the n-6 (omega-6) fatty acid (FA) family. In flaxseed of the traditional variety the main FA is α -linolenic acid (ALA, C18:3), belonging to the n-3 (omega-3) FA family. There are, however, also genetically modified flax varieties with low ALA but high LA contents (5). Petit et al. (26) in their studies reveal a significantly lower LA content and about a 3-fold higher ALA content in the blood plasma of cows receiving a diet including 9.7% of linseed of the traditional variety, compared with those fed a diet containing a similar amount of sunflower seed or cows that did not receive any fat additive.

Results of some studies suggest that dietary fat may influence reproductive indices of cows (31). Diets rich in LA (n-6 family FA) increase the secretion of prostaglandin F_2 (PGF_{2 α}) (26, 30), whereas according to Mattos et al. (20), dietary fatty acids of the n-3 family FA reduce the ovarian and endometrial synthesis of prostaglandin $F_{2\alpha}$, which may contribute to a reduction in embryonic mortality. The results of some other investigations confirm a positive effect of high ALA sources on the fertility of cows (25). A study by Ambrose et al. (2) proves greater presumptive conception and lower pregnancy losses in cows fed linseed (high ALA), compared with those fed a sunflower (high LA) additive.

Most of the existing studies on the use of oil seeds as fat sources compare the effect of seeds of different oil plant species. The aim of the present investigation was to determine the influence of flaxseed of two varieties – a traditional one, with a high ALA content, and a modified one, poor in ALA but rich in LA – on selected blood indices of dairy cows in the transition period and on their reproduction.

Material and methods

The study was carried out on dairy cows during late pregnancy and early lactation. Fifty-six dry multiparous Polish Holstein-Friesian cows were allocated by analogues, 6 weeks prior to expected calving, into 3 groups: one control (C, n = 24) and two experimental (H-ALA and L-ALA), receiving an additive of rolled linseed of two flax varieties (n = 16 heads each). The H-ALA group cows were fed the basal diet enriched with linseed of a traditional variety (LTV, Szafr), characteristic for its high ALA level, whereas the L-ALA group received the same amount of linseed of a genetically modified variety (LMV, Amon), poor in ALA, but rich in LA. Linseed of both varieties contained similar amounts of fat (ca. 42%, DM basis). ALA levels in the fat of the Szafr and Amon varieties were 51.3% and 2.3%, respectively, whereas LA contents in the seeds of these flax varieties were around 16% and 70%, respectively. Flaxseed of both varieties had similar protein concentrations – 22%

and 24% crude protein (DM basis) in the Szafr and Amon varieties, respectively. The animals of all groups were fed in accordance with the IZ-PIB INRA (13) norms. The main ingredients of basal diets were maize silage, grass haylage, and meadow hay, given *ad libitum*. They were given in a mix form (PMR) at the 24:7:69 (DM basis) ratio in the dry period and at the 71:21:8 ratio during lactation. Linseed (substituting part of concentrate) was introduced into the rations in the amounts of 3% and 6% of the diet (DM basis) in the dry and lactation periods, respectively, beginning from the 6th wk prior to parturition until the 17th wk of lactation. In addition, all cows with a daily milk yield of over 20 kg received an appropriate amount of concentrate mixture (0.4 kg concentrate per 1 kg milk over 20 kg milk yield). Cows were housed in tie stalls. For more details on animal feeding in this trial (diet composition and intake) please refer to the paper by Matras et al. (19).

Blood samples were collected 3 weeks before parturition and in the 2nd, 7th, and 17th week of lactation. They were taken from the jugular vein, just before the morning meal. The glucose (GLU), urea (UREA), and total protein (TP) contents, as well as lipid indices, that is non-esterified fatty acids (NEFA), β -hydroxybutyric acid (BHBA), total cholesterol (CHOL), triacylglycerols (TG), and HDL cholesterol fraction, were determined in blood plasma by colorimetric methods, using Biomaxima monotests. Plasma FA profiles, determined for 10 cows per group, were extracted and measured by gas chromatography according to the procedure described in Szumacher-Strabel et al. (33).

All the data were analyzed with the Statistica Software ver. 10 (StatSoft Inc.), using a general linear model (GLM) of the one-way analysis of variance (ANOVA) (32). Tukey's test was applied for multiple comparisons among means, considering $p \leq 0.05$ as significant.

All experimental procedures were approved by the Local Ethical Committee of the Faculty of Biology and Animal Breeding at the University of Life Sciences in Lublin, Poland.

Results and discussion

The blood plasma glucose concentration oscillated around 4.0 mmol l⁻¹, a much higher level than the one regarded as minimum for ruminants (3 mmol l⁻¹) by Whitaker et al. (35). This parameter was not affected significantly by linseed additives. In an experiment conducted by Petit et al. (27) on cows at the beginning of lactation, in which saturated lipids were supplied as an energy booster, a significantly lower blood glucose level was noted in comparison with control animals. In many other studies, however, in which fat was introduced into cow diets in the form of oil seeds, such as LTV (2, 11) or sunflower (2, 26), no changes in the blood glucose level were found.

The plasma urea concentrations for the cows supplemented before calving with linseed (3% of diet DM) were slightly higher in comparison with control animals. The differences, however, were not confirmed statistically, whereas after calving, when linseed was added in the amount of 6% of diet DM, the plasma

urea level increased significantly ($p \leq 0.05$), on average by about 22% and 32% for the H-ALA and L-ALA groups, respectively. A higher level of this plasma index may reflect a lower capture of N in the rumen due to the possibly decreased ruminal microbial protein synthesis, which, according to Tackett et al. (34), may generally be observed when diets are enriched with unprotected fat.

According to Whitaker et al. (35), the desirable plasma concentration of NEFA is below 0.4 mmol l^{-1} in late pregnancy, and it should not exceed 0.7 mmol l^{-1} in early lactation. In the present experiment the average level of this parameter was much below these thresholds. During pregnancy it was equal to 0.31 mmol l^{-1} , and in the 17th week of lactation around 0.3 mmol l^{-1} (Tab. 1), with a peak of 0.37 mmol l^{-1} in

Tab. 1. Blood plasma biochemical indices of pregnant and lactating cows fed flaxseed differing in the α -linolenic acid content

Indices	Week with reference to calving	Treatment			SEM	Influence*	
		C	H-ALA	L-ALA		Flaxseed additive	Flaxseed variety
Glucose, mmol l^{-1}	-3	4.5	4.4	4.5	0.23	ns	ns
	2	4.0	4.4	4.2	0.16	ns	ns
	7	4.1	4.6	3.7	0.19	ns	ns
	17	3.8	4.2	4.0	0.14	ns	ns
Urea, mmol l^{-1}	-3	3.78	4.00	3.97	0.31	ns	ns
	2	2.70 ^b	3.59 ^a	3.75 ^a	0.29	*	*
	7	3.61 ^b	4.01 ^a	4.10 ^a	0.47	*	*
	17	3.09 ^b	3.82 ^{ab}	4.55 ^a	0.39	*	*
Total protein, mmol l^{-1}	-3	50.2	48.8	49.5	4.71	ns	ns
	2	55.1	55.5	51.5	3.52	ns	ns
	7	50.3	54.7	55.6	2.73	ns	ns
	17	51.4 ^b	54.4 ^a	55.8 ^a	3.06	*	ns
NEFA, mmol l^{-1}	-3	0.340	0.307	0.292	0.06	*	*
	2	0.370 ^a	0.226 ^c	0.251 ^b	0.07	*	*
	7	0.312	0.280	0.292	0.04	ns	ns
	17	0.336	0.296	0.281	0.05	ns	ns
BHBA, mmol l^{-1}	-3	0.615	0.583	0.574	0.04	ns	ns
	2	0.584	0.506	0.506	0.03	ns	ns
	7	0.531	0.528	0.490	0.03	ns	ns
	17	0.710 ^a	0.493 ^b	0.596 ^b	0.05	*	*
Total cholesterol, mmol l^{-1}	-3	3.44 ^b	4.09 ^a	4.15 ^a	0.41	*	ns
	2	2.68	3.06	2.93	0.19	*	ns
	7	3.17 ^b	3.90 ^a	3.62 ^a	0.33	*	ns
	17	3.43 ^b	3.13 ^b	4.07 ^a	0.29	*	*
Triacylglycerols, mmol l^{-1}	-3	0.10	0.07	0.08	0.01	ns	ns
	2	0.11	0.12	0.12	0.01	ns	ns
	7	0.10	0.07	0.06	0.01	ns	ns
	17	0.08	0.11	0.07	0.01	ns	ns
HDL-Chol, mmol l^{-1}	-3	2.24 ^b	2.82 ^a	2.89 ^a	0.29	*	*
	2	1.89	2.16	2.10	0.18	*	ns
	7	2.03 ^b	2.81 ^a	2.44 ^{ab}	0.24	*	*
	17	2.42	2.58	2.77	0.31	ns	ns
% HDL	-3	65.2	68.9	69.6	4.33	ns	ns
	2	70.5	70.6	71.7	3.81	ns	ns
	7	64.0 ^b	72.1 ^a	67.4 ^b	4.76	ns	*
	17	70.7 ^{ab}	82.5 ^a	68.0 ^b	5.02	ns	*

Explanations: probability of factor impact * $p \leq 0.05$, ns – non-significant; a, b, c – values in the rows with different letters differ significantly ($p \leq 0.05$)

the control treatment in the 2nd week after calving. Fat supplementation of the diet usually increases NEFA concentrations in blood plasma (11, 12). The results, however, are not univocal (14, 25, 26). In a study carried out by Gonthier et al. (11), who applied 12.6% of LTV in different forms (raw, extruded or micronized) in cow diets, this parameter increased by 40-50%, whereas Petit et al. (26), using 9.7% of raw linseed or sunflower seed, found no significant differences in plasma NEFA between control and experimental treatments. In some investigations, NEFA in the plasma of cows receiving a linseed additive were even lower than in the control group (17, 27, 28), but the differences were not confirmed statistically. In our study, with the 6% linseed supplement (2.5% of fat additive in diet DM), plasma NEFA concentrations in both linseed treatments were lower than in the control group, with significant differences noted in the 2nd week after calving. The lowest level of this blood index was noted in the group receiving LTV. Bobe et al. (4) consider the plasma NEFA concentration a reliable index of the degree of adipose fat mobilization. It seems therefore that the control animals in the present study, with a higher blood NEFA level, may have had a greater lipolysis of adipose tissue than the cows fed the rations with linseed. Comparing the two linseed treatments, a significantly lower blood NEFA concentration in the 2nd week of lactation characterized the cows of the H-ALA group. Similarly, Petit et al. (22) found that cows given LTV (high C18:3 FA) had a significantly lower blood NEFA level than the ones receiving sunflower seeds (low C18:3 FA).

The blood plasma level of β -hydroxybutyric acid (BHBA), which characterizes a short-time energy state of the organism, has often been used to detect ketosis, and concentrations below 1.0 mmol BHBA l⁻¹ have been considered normal (7). In the present study, the average content of BHBA was much below this level, ranging between 0.5 and 0.7 mmol l⁻¹ (Tab. 1). Linseed additives of both varieties did not change it considerably. These results closely correspond with those obtained by Petit et al. (27) in an experiment on transition cows fed diets with 3.3% and 11% of linseed in late pregnancy and early lactation, respectively.

In comparison with the control animals, the average total blood cholesterol concentration during lactation was greater ($p \leq 0.05$) for cows fed diets containing linseed of either variety, and its level reached 3.35, 3.71 and 3.95 mmol l⁻¹ in the control, H-ALA, and L-ALA treatments, respectively (Tab. 1). An increased level of this blood index – a precursor of steroid hormones (9) – may be considered beneficial for ruminants. According to Rabbie and Lean (29), its higher level may contribute to an improved animal health status and reproductive parameters. Fat supplementation usually increases blood cholesterol in ruminants (10, 26), but its effect can be influenced by the fatty acid profile (23, 25). The introduction of a commercial inert fat

Tab. 2. Plasma fatty acid profile (% of total FA) of cows in late pregnancy fed flaxseed differing in the α -linolenic acid content

Indices	Pregnancy			SEM
	C	H-ALA	L-ALA	
C 14:0	1.93 ^a	0.85 ^c	1.12 ^b	0.22
C16:0	16.40	15.71	15.34	0.86
C16:1	1.47 ^a	0.85 ^c	1.09 ^b	0.09
C18:0	19.39 ^a	17.77 ^b	17.81 ^b	1.15
Σ C18:1 trans	1.69 ^b	1.79 ^b	2.32 ^a	0.22
Σ C18:1 cis	8.94	9.45	9.78	0.57
C18:2	41.93	40.07	41.28	2.19
C18:3 n6 c9c12	0.88 ^b	2.49 ^a	2.57 ^a	1.12
C18:3 n3 c9c12c15	1.83 ^c	5.13 ^a	2.58 ^b	1.74
C20:3 n6	3.02 ^a	2.72 ^b	2.44 ^b	0.61
C20:4 n6	2.19 ^b	2.39 ^b	3.06 ^b	0.37
C20:5 n3	0.19 ^c	0.37 ^a	0.23 ^a	0.09
C22:6 n3	0.15 ^b	0.40 ^a	0.39 ^a	0.16
SFA	37.72	34.33	34.27	3.15
MUFA	12.10	12.09	13.18	2.23
PUFA	50.18	53.58	52.55	3.92
omega 6: omega 3	21.85 ^a	7.66 ^c	14.65 ^b	6.73

Explanation: a, b, c – as in Tab. 1

preparation Megalac (based on saturated FA) into the diet in early lactation, elevated blood cholesterol by 64%, whereas LTV increased it by only 24% (25). In another experiment, in which the effect of LTV (high C18:3 FA) was compared with that of micronized soybeans (high C18:2 FA), a significant difference in the blood total cholesterol level (246 vs. 278 mg 100 ml⁻¹) was noted (23). Similarly, in our study in the 17th week of lactation, the cholesterol level was observed to be significantly lower in the H-ALA group (high C18:3 FA) than in the L-ALA group (high C18:2 FA). The results obtained in the present study indicate a higher ($p \leq 0.05$) contribution of the HDL cholesterol fraction in the plasma of cows receiving diets enriched with linseed of the traditional variety in comparison with the control cows and the L-ALA group, whereas Petit et al. (23), who compared LTV and soybean seed additives, did not find any differences in the proportion of cholesterol fractions in total blood cholesterol.

Diets including linseed of both flax varieties decreased ($p \leq 0.05$) plasma concentrations of C14:0, C16:1 and C20:3 n-6 FA, and increased C22:6 n-3 both before calving and during lactation (Tab. 2 and 3). They also increased ($p \leq 0.05$) C18:3 n-6 FA in the blood of cows before calving, in comparison with the control animals (Tab. 2). The flax variety had a particularly great impact on the concentrations and proportion of LA and ALA in blood plasma. The influence of the modified flaxseed on the contents of these two types of FAs during pregnancy and lactation was different. LTV decreased LA and at the same time increased

Tab. 3. Plasma fatty acid profile (% of total FA) of cows in early lactation fed flaxseed differing in the α -linolenic acid content

Indices	Lactation			SEM
	C	H-ALA	L-ALA	
C 14:0	1.12 ^a	0.99 ^b	0.88 ^b	0.13
C16:0	16.05	14.99	14.51	0.92
C16:1	1.13 ^a	0.99 ^b	1.03 ^{ab}	0.07
C18:0	18.15	19.42	18.46	1.03
Σ C18:1 trans	2.28	2.35	2.55	0.38
Σ C18:1 cis	10.33	11.25	11.00	0.71
C18:2	39.39	35.57	39.64	2.05
C18:3 n6 c9c12	1.46 ^b	1.05 ^b	2.36 ^a	0.84
C18:3 n3 c9c12c15	4.47 ^b	7.67 ^a	3.76 ^c	1.32
C20:3 n6	2.90 ^a	2.72 ^a	2.37 ^b	0.47
C20:4 n6	2.17 ^b	2.16 ^b	2.82 ^a	0.59
C20:5 n3	0.34 ^b	0.54 ^a	0.33 ^b	0.19
C22:6 n3	0.23 ^b	0.29 ^a	0.28 ^a	0.13
SFA	35.31	35.40	33.85	2.74
MUFA	13.73	14.59	14.58	1.78
PUFA	50.96	50.01	51.57	3.66
omega 6: omega 3	9.12 ^a	4.88 ^b	10.78 ^a	4.04

Explanation: a, b, c – as in Tab. 1

($p \leq 0.05$) the concentration of ALA, causing a 50% reduction in the omega 6: omega 3 FA ratio ($p \leq 0.05$). The increase in the ALA level noted in this treatment was equal to about 3 percentage points, both in pregnancy (from 1.8 to 4.9%) and lactation (from 5.9 to 8.7%). A similar increment in this FA in blood was noted by Petit and Benchaar (24) in their study on lactating cows (diets supplemented with 9% of linseed).

Pre- and post-calving diets have been reported to affect subsequent reproductive performance (35). Fats in animals' diet may positively influence their reproduction by altering both ovarian follicle and corpus luteum function through improved energy status and by supplying animals with proper amounts and proportion of essential fatty acids of the n-3 and n-6 families, precursors for the synthesis of reproductive hormones, such as steroids and prostaglandins (21). Linoleic acid is converted into arachidonic acid, which is a precursor of dienoic (2-series) prostaglan-

dins (PG), such as $\text{PGF}_{2\alpha}$, whereas ALA is used in the formation of eicosapentaenoic acid (C20:5n-3), the precursor of trienoic (3-series) PG, such as $\text{PGF}_{3\alpha}$ (1). Petit et al. (27) suggest that some embryonic losses in pregnant cows in early gestation may occur because certain embryos are unable to decrease the intrauterine secretion of the luteolytic hormone $\text{PGF}_{2\alpha}$. Therefore, strategies for inhibiting the secretion of $\text{PGF}_{2\alpha}$ may result in increased embryonic survival and pregnancy rates. Mattos et al. (20) proved that diets with fatty acids from the n-3 family fed to periparturient Holstein cows reduced the ovarian and endometrial secretion of $\text{PGF}_{2\alpha}$ and decreased embryonic mortality, whereas diets with a high proportion of n-6 FA (sunflower seed diet) increased the secretion of $\text{PGF}_{2\alpha}$ in the cows' blood and worsened their reproduction indices (26).

Quite a few studies (2, 25) report improved reproductive performance of dairy cows fed diets enriched with seeds of LTV (high ALA content), in comparison with control cows and those receiving oil seeds rich in LA. In a study on dairy cows whose diets were supplemented with LTV, Petit et al. (25) noted increased conception at the first artificial insemination (AI) in comparison with the control group (88% vs. 50%). In an experiment by Ambrose et al. (2), in which LTV or sunflower seeds were added to diets of dairy cows in early lactation, conception rates at first AI were greater with LTV than with sunflower (72.6% vs. 47.5%). A positive influence of LTV on these rates were also confirmed by Petit and Benchaar (24) in their research on dairy cows in the periparturient period. They observed a higher conception rate at first AI in cows fed LTV (54.3%) than in those fed soybean (26.9%), as well as a tendency for lower embryo mortality in the linseed treatment.

In the present study, neither the linseed of the traditional variety nor the modified one had any significant influence on reproductive parameters (Tab. 4). Although the lowest number of semen portions to first AI (insemination index) and the lowest number of days to the first oestrus were noted in the H-ALA group compared with the control cows and the L-ALA group, these differences were not confirmed statistically. The results of the present study correspond closely with those obtained by Fuentes et al. (8), who used extruded linseed and, as in our experiment, did not note any

Tab. 4. Reproductive indices of cows fed flaxseed differing in the α -linolenic acid content

Reproductive indices	Treatment			SEM	Influence*	
	C	H-ALA	L-ALA		Flaxseed additive	Flaxseed variety
Open days	71	70	73	3.82	ns	ns
Reproductive cycle, days	363	359	352	23.9	ns	ns
Days to first oestrus	56	53	55	4.64	ns	ns
Conception%	66	65	69	3.91	ns	ns
Insemination index	1.67	1.58	1.62	0.16	ns	ns

Explanation: * non-significant probability of factor impact

significant change in the reproductive performance of dairy cows. The absence of a significant effect of linseed in our study, as well as in the above-mentioned investigation, may have resulted from a relatively small amount of linseed additive used in these experiments (6% and 5.5% of linseed in diet DM, respectively). In studies that showed a significant improvement in reproductive parameters, either the amounts of linseed were greater, up to 17% (24, 25), or linseed oil was administered in an encapsulated form, which protected unsaturated FAs from biohydrogenation in the rumen (36). This resulted in a substantially increased (up to 5-fold) ALA content in blood plasma, whereas in the current experiment the average increase in this acid level was only about 1.7 times.

Conclusion

The addition of flaxseed of the traditional variety (high omega-3 FA) to the diet of cows in their late pregnancy and early lactation in the amounts of 3 and 6% of diet DM, respectively, increased significantly the ALA content and diminished the omega 6: omega 3 FA ratio in blood plasma. Neither this supplement nor flaxseed of the modified variety (low omega-3 but high omega-6 FA content) had any considerable effect on the fertility of cows.

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