EFFECT OF A PROTEIN-XANTHOPHYLL CONCENTRATE FROM ALFALFA AND OF GENOTYPE AND SEX OF LAMBS ON THEIR BLOOD REDOX PROFILE*

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Abstract

The experiment was conducted on lambs of the Polish Lowland sheep (PLS) and a synthetic BCP line. The redox potential was assessed in blood of lambs with different genotype and sex, which were fed feed mixtures with various contents of a protein-xanthophyll (PX) concentrate from alfalfa (1.5% and 3%). The 1.5% addition of PX concentrate to feed stimulated the response of the antioxidant defence mechanisms of the lambs to a greater extent than the 3% addition. The study showed that sex of the lambs had no significant effect on changes in the redox parameters of blood. The PLS genotype of lambs determined higher values of superoxide dismutase (SOD), total antioxidant potential of plasma (FRAP), malondialdehyde, and vitamin C than the BCP genotype. Along with the growth of the lambs, a descending tendency was observed in SOD and catalase levels, and an ascending tendency in FRAP value.

Key words: lambs, alfalfa, protein-xanthophyll, redox parameters.

The feeding of lambs exerts a significant effect on their rearing performance. It may also contribute to the modification of their metabolism and, consequently, to the improvement of their health status. A protein-xanthophyll (PX) concentrate from lucerne (alfalfa), containing ca. 550 g of total protein and over 1,200 mg of xanthophylls per 1 kg of the preparation, additionally possesses a number of other valuable active compounds, e.g., saponin glycosides (2%-3%), polyphenolic compounds, compounds exhibiting oestrogenic activity (biochanin A, daidzein), as well as vitamins (A, B₁, B₆, B₁₂, C, E, K), provitamins (β-carotene), and mineral compounds (22, 10). The presence of numerous groups of active substances determines the multi-directional pharmacological effects displayed by alfalfa and products made of it (16). Many of these active compounds – polyphenols, vitamins E and C, and β-carotene in particular, apart from immunomodulating properties (9, 14, 19) exhibit antioxidative properties (1). Nevertheless, it is worth emphasising that, besides feeding, changes in the metabolism of animals (including the antioxidative status) may also be affected by a variety of other factors, including: genotype, sex, and age of animal, and by a study period.

The objective of this research was to evaluate the blood redox potential of lambs with different genotype and sex, fattened feed mixtures with various contents of a protein-xanthophyll (PX) concentrate from alfalfa.

Material and Methods

The study was conducted in Experimental Station in Bezek of the University of Life Science in Lublin in two replications in the years 2009 and 2010. Ninety six lambs of two genotypes: the Polish Lowland Sheep (PLS) and synthetic BCP line divided into forty eight animals (24 males and 24 females) within each group were used in the experiment. The animals of each genotype and sex were stratified as analogues into three nutritional groups. Respective groups consisted of thirty two lambs. Group - C the control group was fed the standard full-dose mixture, whereas both experimental group E-1 and E-2 received the same standard mixture but enriched with 1.5% or 3% of protein-xanthophyll (PX) concentrate from alfalfa, respectively, given instead of soybean meal. PX concentrate (Desialis-France Luzerne) was dosed in the full-dose mixture balanced for the respective rearing periods (Table 1) since day 10 of life till weaning (90-100 d of life) (16). In addition, lambs of all groups were receiving a structural additive of meadow hay during the entire period of rearing with mothers. The feeding of lamb mothers was consistent with demands provisioned in the lactation period (17). A feed dose was composed of hay silage (3-4 kg), full-dose feed mixtures (oat grain and wheat bran in the ratio of 3:1, in the quantity of 0.5-0.7 kg/animal), hay (0.8 kg/animal), and straw *ad libitum*. The animals were kept in a sheep shed under identical

conditions, on deep litter, and with free access to drinking water.

In order to analyse redox parameters, blood was sampled from lambs in the 4th, 8th, and 12th week of their life. In blood plasma, superoxide dismutase (SOD) was assayed spectrophotometrically by the adrenaline method, according to Misra (15), at the 320 nm wavelength. The method was modified in order to achieve greater selectivity of transitory reaction products at this wavelength (4). Additionally, catalase (CAT) level was analysed according to Bartosz (4). In the case of the antioxidative system parameters, the ferric reducing ability of plasma (FRAP) was analysed according to Benzie and Strain (5), and vitamin C was determined according to Omaye *et al.* (24). Samples of blood plasma were also analysed for levels of lipid peroxidation products: peroxides (H₂O₂) – according to Gay and Gębicki (12, 13), and malondialdehyde (MDA) – the end product of tissue lipid oxidation – according to Salih *et al.* (25).

The obtained results were analysed statistically using the analysis of variance with the least squares method (SAS 2003) and the following model:

$$Y_{ijklmn} = \mu + \bar{G}_i + \bar{P}_j + \bar{D}_k + \bar{R}_l + e_{ijklmn}$$

where:

- Y_{ijklmn} - value of parameter
- μ - mean of all assays
- G_i - effect of genotype (BCP, PLS)
- P_j - effect of sex (ram, ewe)
- D_k - effect of group (C, E-1, E-2)
- R_l - effect of the year of the study (2009, 2010)
- e_{ijklmn} - random error

Results

Effect of PX concentrate. The values of pro-oxidative and anti-oxidative parameters achieved in blood plasma of the lambs are presented in Table 1-3. The addition of PX concentrate to feed mixtures caused a significant decrease in the activity of superoxide dismutase in group E-1 (4th week of life) and in group E-2 (12th week of life) in comparison to the control group. In the 8th and 12th week of life, in lambs fed the feed mixture with 1.5% content of the PX concentrate the suppressed activity of catalase was also noted, when compared to the control group. The administration of 1.5%-PX caused a significant increase in FRAP, compared to the control animals, which was correlated with a reduced concentration of vitamin E in the 8th week of life. It is worth noting, however, that the longer administration period of the feed mixture with 1.5% PX content triggered a significant increase in vitamin C level, recorded in the 12th week of lambs' life. In contrast, the addition of 3%-PX contributed to a significant decrease in FRAP level only in the first period of administration (4th week). Furthermore, the

addition of 3% PX concentrate resulted in a decreased level of vitamin C (a constituent of FRAP) in the 8th week of lambs' life. The application of both doses (1.5% and 3%) of the PX concentrate to the feed mixtures did not increase the levels of lipid peroxidation products (H₂O₂ and MDA) neither in the 4th nor in the 8th week of lambs' life. Nevertheless, in group E-1, a higher level of MDA was observed in the 12th week of lambs' life, compared to the control group.

Effect of sex, genotype, and the study year.

The analysis of the obtained results demonstrated that sex of the lambs had no impact on the values of most of the analysed parameters, except for MDA, whose higher ($P \leq 0.01$) value was noted in the 4th week of ewes' life. In contrast, animal genotype exerted a significant effect on the activity of SOD ($P \leq 0.05$) and FRAP ($P \leq 0.01$), as well as on the levels of MDA ($P \leq 0.05$) and vitamin C ($P \leq 0.01$). Significantly higher values of these blood parameters were determined in the PLS lambs.

The year of the study was also found to affect the investigated blood parameters. In the experiment conducted in 2010, over the entire fattening period, the level of MDA was significantly higher than the respective value recorded in 2009. In addition, the experiment carried out in 2010 reduced the levels of lipid peroxides (4th and 8th week of life) and vitamin C (4th and 12th week of life) as well as increased the activity of SOD (4th week of life) and CAT (8th week of life).

The observed changes in the redox parameters of lambs' blood, occurring during the growth period of the animals, constitute very interesting results. A descending tendency was noted for the activity of SOD and CAT in the older lambs. In contrast, the FRAP was observed to increase along with the growth of the lambs. Furthermore, when analysing changes in the values of pro-oxidative parameters (H₂O₂ and MDA), their initial increase was observed up to the 8th week of life, which was followed by a decrease noted in the 12th week of lambs' life. In contrast, the age of the lambs did not affect vitamin C level in their blood plasma.

Table 1
Composition of a feed mixture for lambs (%)

Component	I period of fattening			II period of fattening		
	10-30 d of life			Over 30 d of life		
	C	E-1	E-2	C	E-1	E-2
Oat	39	39	39	35	35	35
Wheat	15	15	15	15	15	15
Barley	15	15	15	15	15	15
Wheat bran	15	15	15	15	15	15
Dry beet pulp	8	8	8	8	8	8
Extracted soybean meal	7	5.5	4	11	9.5	8
PX concentrate from alfalfa	-	1.5	3	-	1.5	3
Mineral-vitamin premix – Dolfos for lambs	1	1	1	1	1	1

Table 2
Pro-oxidative and antioxidative parameters in blood in the 4th week of lambs' life

Parameter		Genotype (G)		Sex (S)		Experimental group (E)			Study year (Y)		Effect of interaction
		BCP n=48	PLS n=48	Ewes n=48	Rams n=48	Control (C) n=32	1.5% PX (E- 1) n=32	3% PX (E-2) n=32	2009 n=48	2010 n=48	
SOD U mL ⁻¹	LSM	99.15	94.66	97.70	96.11	112.63 ^a	85.29 ^b	92.79 ^{ab}	64.49**	112.31**	GxD**
	SE	9.39	7.39	8.96	7.88	11.68	7.58	10.99	1.37	9.41	
CAT U mL ⁻¹	LSM	5.52	5.44	5.48	5.48	5.86	5.44	5.15	5.80	5.16	ns
	SE	0.38	0.31	0.34	0.36	0.46	0.41	0.41	0.31	0.37	
FRAP μmol L ⁻¹	LSM	58.47**	75.84**	69.92	64.39	77.02 ^A	75.85 ^A	58.59 ^B	58.71**	75.59**	GxR**
	SE	3.18	4.40	4.49	3.46	2.55	5.75	4.07	3.36	4.20	
VIT.C mg L ⁻¹	LSM	0.25	0.28	0.26	0.27	0.27 ^{ab}	0.29 ^a	0.24 ^b	0.28*	0.25*	GxR**
	SE	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	
H ₂ O ₂ μmol L ⁻¹	LSM	4.09	3.54	3.90	3.71	3.48	4.84	3.11	4.54**	3.07**	ns
	SE	0.29	0.28	0.31	0.27	0.31	0.37	0.29	0.23	0.30	
MDA μmol L ⁻¹	LSM	0.66*	0.75*	0.76**	0.65**	0.79	0.60	0.73	0.27**	1.14**	ns
	SE	0.06	0.08	0.08	0.06	0.09	0.07	0.11	0.01	0.05	

a, b - means within the row with no common superscript differ significantly at P≤0.05 (comparison to control group I)

A, B - means within the row with no common superscript differ significantly at P≤0.01 (comparison to control group I)

ns: not significant (P>0.05); * (P≤0.05); ** (P≤0.01)

SOD - superoxide dismutase, CAT - catalase, FRAP - total antioxidant potential of plasma, VIT C - vitamin C, H₂O₂ – peroxides, MDA – malondialdehyde

Table 3
Pro-oxidative and antioxidative parameters in blood in the 8th week of lambs' life

Parameter		Genotype (G)		Sex (S)		Experimental group (E)			Study year (Y)		Effect of interaction
		BCP n=48	PLS n=48	Ewes n=48	Rams n=48	Control (C) n=32	1.5% PX (E-1) n=32	3% PX (E-2) n=32	2009 n=48	2010 n=48	
SOD U mL ⁻¹	LSM	80.47	72.01	76.34	76.14	86.86	74.42	67.45	71.31	81.18	ns
	SE	10.43	2.01	5.16	9.77	14.98	4.45	5.65	1.21	10.75	
CAT U mL ⁻¹	LSM	3.90	3.47	3.52	3.86	4.58 ^a	3.03 ^b	3.46 ^{ab}	2.35**	5.03**	ns
	SE	0.41	0.45	0.39	0.47	0.59	0.43	0.52	0.15	0.51	
FRAP μmol L ⁻¹	LSM	50.88* *	66.89* *	59.40	58.36	48.36 ^B	74.24 ^A	54.04 ^A B	71.13* *	46.63* *	GxR** DxR**
	SE	4.09	2.66	3.63	3.68	2.93	5.43	3.20	3.16	3.18	
VIT.C mg L ⁻¹	LSM	0.23**	0.30**	0.27	0.26	0.34 ^A	0.23 ^B	0.22 ^B	0.28*	0.25*	DxR**
	SE	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	
H ₂ O ₂ μmol L ⁻¹	LSM	4.65*	4.13*	4.37	4.42	4.21 ^{AB}	5.15 ^A	3.81 ^B	4.77*	4.02*	GxR**
	SE	0.21	0.23	0.19	0.25	0.29	0.26	0.20	0.19	0.24	
MDA μmol L ⁻¹	LSM	0.80	0.82	0.81	0.82	0.83	0.84	0.78	0.25**	1.37**	ns
	SE	0.08	0.09	0.08	0.09	0.10	0.11	0.10	0.02	0.03	

Symbols as in the footnote to Table 2.

Table 4
Pro-oxidative and antioxidative parameters in blood in the 12th week of lambs' life

Parameter		Genotype (G)		Sex (S)		Experimental group (E)			Study year (Y)		Effect of interaction
		BCP n=48	PLS n=48	Ewes n=48	Rams n=48	Control (C) n=32	1.5% PX (E-1) n=32	3% PX (E-2) n=32	2009 n=48	2010 n=48	
SOD U mL ⁻¹	LSM	20.83*	22.95*	21.64	22.14	34.20 ^A	17.54 ^B	13.94 ^B	21.76	22.02	ns
	SE	1.34	1.51	1.49	1.44	1.00	0.60	0.48	1.47	1.46	
CAT U mL ⁻¹	LSM	2.55	2.38	2.39	2.55	2.59 ^{AB}	1.60 ^B	3.21 ^A	2.43	2.51	GxP*
	SE	0.18	0.14	0.15	0.16	0.16	0.10	0.18	0.16	0.16	
FRAP μmol L ⁻¹	LSM	123.82	132.08	131.91	123.98	129.49 ^{ab}	120.71 ^b	133.64 ^a	128.55	127.34	ns
	SE	2.76	3.26	3.94	2.15	3.23	4.16	3.75	3.35	2.93	
VIT.C mg L ⁻¹	LSM	0.21	0.20	0.20	0.20	0.17 ^B	0.25 ^A	0.18 ^{AB}	0.28**	0.13**	GxR**
	SE	0.02	0.02	0.01	0.02	0.01	0.03	0.02	0.02	0.00	
H ₂ O ₂ μmol L ⁻¹	LSM	2.86	2.75	2.91	2.70	2.75 ^{AB}	2.41 ^B	3.26 ^A	2.74	2.87	ns
	SE	0.12	0.13	0.13	0.12	0.15	0.12	0.15	0.13	0.13	
MDA μmol L ⁻¹	LSM	0.20	0.21	0.21	0.20	0.17 ^B	0.25 ^A	0.19 ^B	0.12**	0.28**	GxR*
	SE	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.01	

Symbols as in the footnote to Table 2.

Discussion

In a properly functioning organism, a balance exists between oxidative processes and defence mechanisms. The oxidative agents are products of lipid peroxidation (peroxide radicals, malondialdehyde). In turn, the antioxidative system is constituted by endogenous and exogenous substances being suppressive against radicals (RFT), including enzymes: SOD, glutathione peroxidase (PG_X), and CAT, as well as many low-molecular antioxidants, *e.g.* ascorbic acid, bilirubin, uric acid, urea, glutathione, and tocopherol, that build the FRAP (4). The evaluation of the oxido-reductive (redox) status may refer to both the physiological and pathological state of the body or may be used to investigate effects of antioxidants administered with a diet on that status (2). The significantly lower – when compared to the control group – activities of SOD and CAT, and increased levels of FRAP and vitamin C in blood plasma of the lambs (especially those from E-1 group) fed the feed mixture with 1.5% addition of the PX concentrate is a positive effect. Presumably, the addition of the PX concentrate, characterised by a high antioxidative potential (23), perfectly replenishes shortages of low-molecular antioxidants, thus stimulating mechanisms of the antioxidative defence of lambs' body. Results of the research conducted by Deger *et al.* (8) prove that the application of antioxidants in sheep feeding may significantly suppress the mechanisms of formation of radicals and stress owing to the stimulation of antioxidative reactions. As reported by Barham *et al.*

(3), the enhanced activity of SOD and CAT is observed upon the generation of high quantities of hydrogen peroxide in the body, which was, however, not confirmed in the present study. A difficult to interpret exception was the increased level of MDA in the final fattening period of the lambs receiving 1.5% of the PX concentrate in the feed mixture. It is likely that, apart from a numerous groups of bioflavonoids present in alfalfa, the stimulation of the antioxidative potential of blood plasma of the lambs is additionally connected with selected proteins. A few reports are available, which point to the antioxidative properties of proteins extracted from various plants, including alfalfa leaves (18, 20, 27). Alfalfa proteins display the capability for electrons or hydrogen donation, and thereby are able to neutralise peroxide, hydroxyl, and DPPH radicals (28). The high radical-scavenging activity of alfalfa proteins additionally enhances the antioxidative effects of active compounds being its constituents. A research by Fu (11) conducted on mice demonstrated that proteins of alfalfa leaves were reducing the concentration of MDA and were increasing the FRAP level. Positive effects of applying alfalfa silage were shown in dairy cows feeding, where contents of non-enzymatic antioxidants (vitamin E) were increasing in both blood plasma and milk (6). The stimulating effect on the antioxidative status was also reported upon administering a PX concentrate from alfalfa in feed mixtures for turkey toms (23) and turkey hens (7). The results of our experiments showed no effect of lambs' sex and a significant effect of their genotype on the analysed blood markers of the redox status. No effect of sex differences of healthy

sheep on the levels of redox parameters (MDA, SOD, CAT, GPX) was demonstrated by Nazifi *et al.* (21). Unfortunately, the available literature provides scarce information on the effect of sex and genotype of sheep on changes in oxido-reducing parameters of their blood; hence, confrontation of the obtained results with findings of other authors is difficult. Analyses of experiments conducted on the lambs demonstrated that the activities of SOD and CAT diminished along with the growth of the animals. When investigating the activity of SOD in blood of sheep, Sgorlon *et al.* (26) showed an ascending tendency of this parameter with the age of animals. In turn, the authors firstly observed an increase, followed by a decrease in the level of MDA with the age of the animals, which is consistent with the results of our study.

In summary, considering the results of the stimulation of the antioxidative defence response in lambs, and the economic aspect, the application of 1.5% addition of the protein-xanthophyll (PX) concentrate from alfalfa seems to be more advisable. The sex of the lambs had no significant effect on changes in redox parameters of blood. In turn, the PLS genotype of lambs was found to determine an increase in the values of the analysed parameters (SOD, FRAP, MDA, vitamin C), in comparison to the values noted for the BCP line lambs. A descending tendency in SOD and CAT activities, as well as an ascending tendency in FRAP level were noted during the growth of the animals.

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