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Blood metabolic profile of goat kids fed a diet supplemented with alfalfa protein-xanthophyll (PX) concentrate during rearing with their dams

Anna Szymanowska#, Tomasz M. Gruszecki, Anna Miduch

University of Life Sciences in Lublin, Faculty of Biology, Animal Sciences and Bioeconomy, Department of Small Ruminant Breeding and Agricultural Advisory
ul. Akademicka 13, 20-950 Lublin, Poland; #e-mail: anna.szymanowska@up.lublin.pl

The research objective was to assess the effect of dietary supplementation with alfalfa protein-xanthophyll (PX) concentrate on haematological and biochemical parameters of the blood of goat kids. The study was conducted on 82 goat kids of the Saanen breed, reared with their dams. From 30 days of age the experimental group of kids (41 goats) received a feed mixture supplemented with 3% protein-xanthophyll PX concentrate, while the control was fed a standard, unsupplemented diet. Prior to the start of the experiment (29 days of age) and after its completion (121 days), blood was collected from the kids to determine haematological and biochemical indices. The results and their analysis indicate that inclusion of 3% alfalfa protein-xanthophyll (PX) concentrate in the kids' diet positively affected the haematological and biochemical parameters of the blood. A higher level of erythrocytes, haemoglobin and haematocrit was noted, together with a decrease in the lymphocyte and monocyte count. The blood of goat kids receiving the PX concentrate-supplemented diet had significantly reduced content of total cholesterol and triglycerides, as well as low-density lipoproteins (LDL). The blood of the experimental kids showed a significantly higher level of total protein and urea. The alfalfa concentrate significantly diminished the activity of alanine and aspartate aminotransferases. Protein-xanthophyll (PX) concentrate of alfalfa can be used as a phytobiotic providing an excellent source of protein, vitamins and minerals essential for the proper function of the animal organism.

KEY WORDS: alfalfa protein-xanthophyll (PX) concentrate / goat kids / blood parameters

Laboratory blood tests play an important role in the diagnosis of animal health. They enable assessment of homeostasis in the body of animals under the influence of various external environmental factors. The results provide precise information on the health condition of the animal and the functions of individual organs, indicating potential disorders

and facilitating determination of organ efficiency and the directions of metabolic transformations in the body [14].

Due to the ban on antibiotic growth promoters in the diet of farm animals, it became necessary to replace pharmaceuticals with natural additives which would have beneficial effects on animal health and production results, as well as ensuring production of high quality food. Pre- and probiotics, feed enzymes and herbs are most frequently proposed as safe feed additives [19]. As plant-derived feed additives (herbs) are rich in biologically active substances, they may have positive, multi-faceted effects in animal husbandry, in both prophylaxis and treatment [9]. Alfalfa can play a significant role in this context. It is an important perennial plant which is highly suitable for silage, drying and direct feeding. It is a rich source of protein, vitamins and numerous microelements. In France, scientists have developed a method of extracting protein-xanthophyll concentrate (PX - Protéine-Xanthophylle) from alfalfa. Its annual production is about 4,000 tonnes. The concentrate contains 55-60% total protein with a rich amino acid composition, polyunsaturated fatty acids and xanthophyll—a natural yellow pigment (1,200-2,200 mg/d^3) [4]. It also contains large amounts of chlorophyll, highly bioavailable iron and other important minerals essential for physiological processes. Owing to these features, PX concentrate could find wider application as an additive in animal feeds [13].

The aim of the research was to determine the influence of alfalfa protein-xanthophyll (PX) concentrate on haematological and biochemical parameters of goat kid blood. The intensity and tendencies of any changes observed may indicate the influence of the experimental factor.

Material and methods

The experiment was conducted twice on 82 Saanen goat kids (1st year – 38 animals, 2nd year – 44 animals) of both sexes (43 males and 39 females). During the study the goat kids were kept in standardized habitat and dietary conditions, under continual zootechnical and veterinary control. The goat kids were reared with their dams. During the first two weeks of age they were fed only on their mothers' milk. From the 15th day, solid feeds such as crushed oat grain or hay were included in their daily diet in addition to milk. At the age of 30 days the animals were divided into two feeding groups: control (41 animals), receiving a standard compound feed without additives, and experimental (41 animals), receiving a feed with 3% supplementation with alfalfa protein-xanthophyll (PX) concentrate, which partially replaced soybean extraction meal (Tab. 1). The goat kids consumed the compound feeds prepared for the experimental and control groups for 90 days.

In two periods, i.e. at the start of the experiment (when the goat kids were 29 days old) and at its completion (at 121 days of age), blood was drawn from the jugular vein of all animals.

Table 1
Component composition (%) and nutritional value of compound feed for goat kids during rearing

Specification	Experimental group	Control group
Oat	35	38
Wheat	15	15
Barley	15	15
Wheat bran	15	15
Dried sugar beet pulp	8	8
Soybean meal	5	8
PX protein concentrate	3	-
Mineral and vitamin supplement	1	1
Total protein (g/kg)	114	113
Net energy (MJ/kg)	6.78	6.74

The following blood cell parameters were determined in the whole blood samples: red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), white blood cells (WBC), lymphocytes (LYM), granulocytes (GRA), monocytes (MON), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW) and mean platelet volume (MPV).

The following biochemical markers were identified in the serum: cholesterol (CHOL) and its fractions—triglycerides (TG), LDL and HDL, total protein (TP), urea (UREA), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The haematological assays were performed at a certified laboratory with a BS 400 automatic haematology analyser, and the biochemical parameters were tested with the Prestige 24i analyser.

The results were subjected to multivariate analysis of variance by the least squares method using STATISTICA v. 9.1.3. [16]. The tables present the least square means (LSM) of the parameters tested and the standard error (SE). The significance of differences between means was determined by Duncan's test at significance levels of $p \leq 0.01$ and $p \leq 0.05$.

The following linear model was used in the statistical calculations:

$$Y_{ijkl} = \mu + G_i + R_j + P_k + (GR)_{ij} + (GP)_{ik} + e_{ijkl}$$

where:

Y_{ijkl} – level of the examined parameter
μ – arithmetic mean for the population
G_i – effect of the i^{th} group (i =1, 2)
R_j – effect of the j^{th} year of the study (j = 1, 2)
P_k – effect the k^{th} sex (k = 1, 2)
$(GR)_{ij}$ – effect of the group x year interaction (i, j = 1, 2)
$(GP)_{ik}$ – the effect of the group x sex interaction (i, k = 1, 2)
e_{ijkl} – sampling error

The statistical analysis revealed no influence of the year or sex on the parameters tested. The interactions proved insignificant in all cases; for this reason only the values for the groups of animals (the control and the experimental one) are shown in the tables.

Results and discussion

The haematological parameters of the goat kid blood are presented in Table 2. The analysis of the results showed that the goat kids from the experimental group had higher RBC ($p \leq 0.01$), HCT ($p \leq 0.01$) and HGB ($p \leq 0.05$) than the animals in the control group. The levels of erythrocytes, haemoglobin and haematocrit increased by about 13%, 4.3% and 30%, respectively. The red blood cells of the goat kids that received the PX additive presented significantly ($p \leq 0.01$) lower haemoglobin saturation (MCH) and concentration (MCHC) than those of the goat kids in the control group. These values were lower by 29% and 20%, respectively. The MCV and MPV levels were similar in the two dietary groups. The improvement in the red blood cell indices in the experimental group may have resulted from the rich composition of the PX concentrate (amino acids, iron, calcium, copper and vitamins), high bioavailability of iron in erythropoiesis, and better absorption of elements (iron and copper) involved in erythropoiesis [17]. Similar tendencies have been noted in studies on the use of PX in lamb [5] and turkey [6] fattening.

Alfalfa is mainly used as an animal feed, but as it has numerous medicinal properties it is used in folk medicine as well. Owing to its abundance of vitamins, mineral salts and chlorophyll, as well as its alkalizing properties, it has been used to treat digestive, circulatory and respiratory disorders. According to Głowniak et al. [8], the results of multi-faceted research on the properties of alfalfa will lead to greater appreciation for the plant and to its wider application in human medicine [18, 22].

The values obtained for the white blood cell indices of each of the dietary groups were within reference limits [21]. The analysis of the blood profile showed that the ani-

Table 2
Indices of goat kid blood

Feature		Group	
		control	experimental
Red blood cells			
red blood cells (10^{12} l^{-1}) (RBC)	LSM SE	9.80** 0.17	11.07** 0.47
haemoglobin (mmol l^{-1}) (HGB)	LSM SE	6.32* 0.10	6.59* 0.12
haematocrit (l l^{-1}) (HCT)	LSM SE	0.19** 0.007	0.25** 0.01
Mean corpuscular volume (fl) (MCV)	LSM SE	30.32 0.33	29.52 0.71
mean corpuscular hemoglobin (f mol^{-1}) (MCH)	LSM SE	1.28** 0.04	0.91** 0.03
mean corpuscular hemoglobin concentration (mmol l^{-1}) (MCHC)	LSM SE	36.17** 1.31	28.82** 1.60
red cell distribution width (%) (RDW)	LSM SE	15.73* 0.26	17.07* 0.40
mean platelet volume (μm^3) (MPV)	LSM SE	3.60 0.02	3.54 0.03
White blood cells			
white blood cells (10^9 l^{-1}) (WBC)	LSM SE	9.04 0.30	9.86 0.42
lymphocytes (%) (LYM)	LSM SE	62.27** 1.73	47.55** 1.96
monocytes (%) (MON)	LSM SE	4.97** 0.21	3.77** 0.28
granulocytes (%) (GRA)	LSM SE	32.06** 1.76	48.68** 1.93

**$p \leq 0.01$, * $p \leq 0.05$

mals in the experimental group had a slightly higher but statistically insignificant white blood cell count (WBC). However, the leukogram revealed pronounced differences. In comparison with their counterparts from the control group, the blood of the goat kids in the experimental group contained about 24% fewer lymphocytes and monocytes and

52% more granulocytes. As there are no published results of studies concerning the use of PX concentrate in the diet of goat kids, we must refer to analogous research on other farm animals. Similar research conducted on monogastric animals did not confirm the significant influence of supplementation of the feed ration with PX concentrate on leukocyte levels or the leukogram [5,12].

The results of the present research may indicate good health in the experimental animals, which would suggest that measures should be taken to promote the advantages of the PX concentrate as a supplement in the diet of young ruminants with beneficial effects on the growing animal.

Table 3 presents lipid components in the goat kid blood serum. The study results confirmed statistically significant ($p \leq 0.01$) changes, i.e. a reduction in total cholesterol levels in the blood of the experimental animals by about 15% and triglyceride levels by 24% in comparison with the control. In the case of high density lipoproteins (HDL), no effects of the dietary factors were noted. In both feeding groups HDL was at similar levels, amounting to 1.74 mM l^{-1} and 1.79 mM l^{-1} in the control and experimental groups, respectively. On the other hand, the PX concentrate differentiated levels of low density lipoprotein (LDL). The LDL concentration significantly ($p \leq 0.05$) decreased (0.32 mM l^{-1}) in the serum of the experimental goat kids. The reduction of the LDL fraction, referred to as 'bad cholesterol', is highly advantageous in the blood plasma. The main function of low density lipoproteins is the transport of cholesterol to cells, e.g. epithelial cells of arterial vessels, where it accumulates to form atherosclerotic plaque.

An experiment using 1.5% and 3% shares of alfalfa protein-xanthophyll (PX) concentrate in compound feeds for turkeys showed a significant influence of the concen-

Table 3
Lipid components (mmol l^{-1}) in goat kid blood serum

Feature		Group	
		control	experimental
Total cholesterol (CHOL)	LSM SE	2.49** 0.10	2.13** 0.11
Triglycerides (TG)	LSM SE	0.37** 0.02	0.28** 0.01
HDL-cholesterol	LSM SE	1.74 0.20	1.79 0.12
LDL-cholesterol	LSM SE	0.41* 0.13	0.32* 0.20

**$p \leq 0.01$, * $p \leq 0.05$

trate on some parameters of the plasma lipid profile. The analyses revealed a significant decrease ($p \leq 0.05$) in total cholesterol and triacylglycerol levels in the experimental group as compared to the control [10, 12]. The effect obtained may have been due either to better utilization of lipid components or to the fact that the addition of some plants, chiefly herbs, increases secretion of bile acids, and almost half of the cholesterol converted into bile acids is excreted with the faeces [2, 5].

Owing to its numerous biologically active components, alfalfa can contribute to the activation of lipid metabolism in animals [15]. Hypocholesterolaemic activity is ascribed to the saponins in alfalfa. The use of alfalfa in animal diets may contribute to a decrease in cholesterol levels without affecting the HDL cholesterol fraction [1]. We can also observe a decrease in cholesterol absorption in the intestines and an increase in steroid and bile acid secretion.

According to Borys [3], excess cholesterol in the human diet can lead to disorders in lipid metabolism, resulting in increased cholesterol concentration in the blood plasma and a higher frequency of cardiovascular disease. An excessive TG concentration (hypertriglyceridaemia), like elevated cholesterol levels, may be the cause of many serious diseases.

Table 4 presents the biochemical parameters analysed (total protein, urea and the enzymes ALT, AST and LDH). The results clearly indicate a highly significant ($p \leq 0.01$) increase in protein levels in the goat kids in the experimental group. The mean values for this parameter were 12% higher than in the control. A similar tendency was observed in the case of urea, as its content was 10% higher than in the control, at 7.37 mM l^{-1}.

Wang et al. [20] obtained similar results using dried alfalfa in the diet of adult sheep, demonstrating a significant ($p \leq 0.05$) increase in the total protein level. According to the authors, alfalfa intensifies protein synthesis by improving conditions in the rumen [20]. Other studies, however, have not confirmed any significant influence of alfalfa hay on total protein content in sheep serum [7].

The present study also analysed the activity of alanine (ALT) and aspartate (AST) aminotransferases and lactate dehydrogenase (LDH). The level of ALT activity in the experimental group was reduced by 12% and that of AST by 14%. No differences were observed between groups in the activity of lactate dehydrogenase (LDH), which was about 900 U l^{-1} in both groups.

The results for the activity of alanine and aspartate aminotransferases are confirmed by the results of an experiment conducted on young fattening cattle. A compound feed supplemented with a 3% addition of alfalfa PX concentrate significantly reduced the activity of both the ALT and AST enzymes by about 28% [11].

In conclusion, supplementation of compound feeds for goat kids with a 3% addition of alfalfa protein-xanthophyll concentrate has a beneficial effect on the haematological and biochemical blood profile. Higher erythrocyte, haemoglobin and haematocrit levels were observed, with a simultaneous decrease in lymphocyte and monocyte counts. Levels of total cholesterol, triglyceride and low density lipoprotein

Table 4
Biochemical indices of goat kid blood serum

Feature		Group	
		control	experimental
Total protein (g l^{-1}) (TP)	LSM SE	59.64** 0.87	66.75** 0.67
Urea (mmol l^{-1}) (UREA)	LSM SE	6.69* 0.22	7.37* 0.17
Alanine aminotransferase (U l^{-1}) (ALT)	LSM SE	30.09** 0.87	26.52** 0.81
Aspartate aminotransferase (U l^{-1}) (AST)	LSM SE	160.50** 3.31	138.07** 3.66
Lactate dehydrogenase (U l^{-1}) (LDH)	LSM SE	934.30 25.95	877.32 22.82

**$p \leq 0.01$, *$p \leq 0.05$

(LDL) were significantly reduced in the blood of the goat kids fed the compound feed supplemented with the PX concentrate. The animals in the experimental group had significantly higher total protein and urea levels. The alfalfa-based product significantly diminished the activity of alanine and aspartate aminotransferases. Protein--xanthophyll (PX) alfalfa concentrate can be regarded as a phytobiotic providing a rich source of protein, vitamins and minerals essential for the proper function of the animal organism.

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