THE EFFECT OF DIETS WITH VARIED PROPORTIONS OF ARGININE AND LYSINE ON BIOCHEMICAL AND ANTIOXIDANT STATUS IN TURKEYS*

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Abstract

The aim of the study was to determine the effect of two proportions of arginine (95% and 105%) relative to lysine (Lys), where Lys content in the diet is in accordance with NRC (1994) recommendations or 10% higher, on the metabolism, antioxidant status, and growth performance of turkeys. The experiment had a 2 x 2 factorial design with two levels of Lys and Arg. The diets with a low level of Lys were according to the NRC (1994) requirements. In the diets with a high level of Lys, the content of Lys was increased by 10% relative to the low level Lys. The two Arg levels in the experimental diets were determined so as to provide 95% and 105% Arg relative to the content of dietary Lys. An increase in the amount of Lys in the diet of turkeys by 10% relative to NRC nutritional recommendations (1994) was not shown to improve growth performance, but had beneficial effects on the metabolism and antioxidant status of the birds, as evidenced by the improvement of hepatic indices (reduction of AST and ALT activity at 9th week of life) and renal indices (reduction of UREA at 9th week of life and reduction of TP and increase level of ALB levels at 16th week of life), as well as an increase in the level of glutathione with strong antioxidant properties at 16th week of life. In comparison to the lower level of Arg in the diet, an increase in the amount of this amino acid to 105% Lys did not improve growth performance, metabolism, or antioxidant status. An Arg level of 95% Lys can be used in a diet for turkeys containing 10% more Lys than the level recommended by the NRC (1994).

Key words: turkey, amino acid, blood, antioxidant, metabolism

The use of diets with an appropriate amino acid profile in the nutrition of turkeys plays an important role in ensuring proper metabolism and allows for the optimal use of their growth potential (Zampiga et al., 2018; Al-agawany et al., 2020; Jankowski et al., 2020 a, b; Ognik et al., 2021 a, b). The genetic potential for growth in turkeys can be fully exploited only when they are fed an appropriate diet. In fast-growing turkeys it is crucial to meet the requirements for amino acids, which are involved in the synthesis of structural proteins and catalyse numerous biochemical reactions. Strictly essential amino acids that must be included in the diet of poultry raised for meat include lysine (Lys), arginine (Arg), methionine (Met) and threonine (Thr), as birds are not able to synthesize them (Zampiga et al., 2018; Handique et al., 2019; Ognik et al., 2021 a). As natural components of poultry feed (maize, wheat, and soybean meal) contain small quantities of these amino acids, they must be added to the diet in pure synthetic form, with estimated digestibility of about 100% (Handique et al., 2019).

According to Urdaneta-Rincon and Leeson (2004) and Liao et al. (2015), Lys is involved in lipid metabo-

lism, taking part in beta-oxidation of fatty acids by promoting synthesis of L-carnitine. Moreover, Lys is responsible for synthesis of nitric oxide (NO), which takes part in the immune response and improves the body's antioxidant status (Ruan et al., 2019). A Lys deficiency can result in an increase in the cholesterol level in the blood, fatty liver, and excessive abdominal fat (Zampiga et al., 2018). Jia et al. (2019) showed that higher intake of Lys in the diet than that recommended by the NRC (1994) can negatively affect growth performance in birds. Arg is a precursor of creatine, polyamines, proline, glutamate, citrulline and agmatine. It takes part in NO synthesis and thus is responsible for the body's immune and antioxidant response. Arg stimulates secretion of insulin and insulin-dependent growth factor, which determine growth processes in poultry (Wu and Morris, 1998; Chamrusspollert et al., 2002; Xu et al., 2018; Khatun et al., 2018). An Arg deficiency in the diet of broilers negatively affects growth performance (Fouad et al., 2013). There are also reports indicating that excessive Arg in the diet can also negatively affect the growth performance of poultry raised for meat, probably due to an

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incorrect proportion of Lys and Arg (Khajali and Wideman, 2010).

The level of Lys, as the first amino acid limiting the nutritional value of feed, is used as a reference point for balancing other amino acids in the diet of birds (Hung et al., 2020). The available literature, however, indicates that due to the similar chemical structure of Lys and Arg, antagonistic reactions may occur between them. Therefore it is extremely important to properly balance these amino acids in the diet, as an excess of one of them can result in a deficiency of the other, leading to unfavorable changes in metabolism and thus negatively affecting growth (Silva et al., 2012). According to the NRC (1994) guidelines, diets fed to turkeys growing in the first 4 weeks of rearing should contain 1.60% Lys and 1.60% Arg, compared to 1.76% and 1.80%, respectively, recommended by breeding companies (BUT, 2013). Moreover, the NRC (1994) recommends that the Arg level in the diet should be 90–100% of the Lys content. According to Silva et al. (2012), however, these recommendations were based on the results of studies carried out several decades ago, when the poultry was not reared as intensively as it is today. For this reason breeding companies (BUT, 2013) recommend a higher Arg level (102–105%) in the diet of fast-growing turkeys. Such large discrepancies between the nutritional recommendations recommended by the NRC (1994) and the current recommendations of breeding companies (BUT, 2013) cause numerous controversies, therefore it is necessary to conduct further research to determine the unequivocal optimal level and quantitative relationship between Lys and Arg in compound feed.

The research results published so far show that the proportion of Arg and Lys in the diet of birds may have a significant impact on growth parameters as well as their immune response, metabolism and antioxidant status (Zampiga et al., 2018; Jankowski et al., 2020 a, b; Ognik et al., 2020, 2021 a, b; Konieczka et al., 2021). The results of our previous studies have shown that increasing the Met level (45% Lys) has a positive effect on the growth parameters and the immune status of slaughter turkeys (Jankowski et al., 2020 b), which clearly suggests that the recommendations of the NRC (1994) for this amino acid should be modified in order to ensure the optimal functioning of the organism of slaughter birds. It is assumed that increasing the Met level entails the necessity to increase the level of Lys and Arg in poultry nutrition and to establish their most appropriate proportions in relation to each other. This assumption seems to be supported by our numerous studies that have shown that in growing turkeys fed diets with low Lys content meeting NRC (1994) requirements, the level of Arg cannot be limited to 90% Lys, as this may negatively affect the growth and antioxidant status of turkeys, whereas increasing the level of Arg to 110% Lys can result in increased protein nitration (Jankowski et al., 2020 a, b; Ognik et al., 2020, 2021 b). In addition, the results of our other research indicated that by feeding turkeys

a diet with high Lys content close to the recommendations of breeding companies (BUT, 2013), the proportion of Arg can be reduced to 90% Lys with a Met level of 45% Lys, as these proportions have no negative effect on growth performance, and metabolism and the antioxidant system functions efficiently (Jankowski et al., 2020 b). Moreover, our other studies have shown that the use of a 10% higher level of Lys than recommended by the NRC (1994) in combination with a higher level of Arg (105% Lys) favorably stimulates the immune system of turkeys and protects proteins and DNA from damage caused by oxidative and nitration processes (Ognik et al., 2021 b), as well as improves the physiological status of the intestines of birds, e.g. due to a positive impact on their microbiome (Konieczka et al., 2021).

As our earlier studies clearly showed that the Met level should be higher than recommended by the NRC (1994), it was assumed that an additionally appropriately selected proportion of Arg: Lys can have a positive effect on the metabolism and antioxidant status of turkeys. Therefore, the aim of the study was to determine the effect of two proportions of arginine (95% and 105%) relative to lysine (Lys), where Lys content in the diet is in accordance with NRC (1994) recommendations or 10% higher, on the metabolism, antioxidant status, and growth performance of turkeys.

Material and methods

The study presented in this research paper is part of a large research project to determine the effects of different levels and proportions of methionine, lysine and arginine in nutrition on many different aspects of the biological response of turkeys in order to establish their most optimal relationship between these amino acids in the feed. Therefore, both the design of the experiment, the research procedures used and some physiological results have been published already in the study of Ognik et al. (2021 b) and Konieczka et al. (2021).

Experimental design and diets

The protocol for the study was approved by the Local Ethics Committee (no 82/2017), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU.

A total of 576 one-day-old female Hybrid Converter turkeys were used in the experiment. They were purchased at a local hatchery and assigned at random to four feeding groups. Each group consisted of 8 replications (pens), with 18 birds in each pen. Each pen had an area of 4 m². The birds were reared in a floor pen system with wood shavings for litter. The microclimate conditions were the same for all birds and were automatically adjusted to their age according to the recommendations of Hybrid Turkeys (BUT, 2013).

The turkeys were raised until the age of 16 weeks and fed *ad libitum* isocaloric diets of wheat and soybean

meal with varied levels of Lys and Arg. The experiment had a completely randomized 2 x 2 factorial design with two levels of Lys (low and high – L_L and L_H) and two levels of Arg (low and high – A_L and A_H). The diets with a low level of Lys (L_L) were designed to provide 1.60, 1.50, 1.30 and 1.00 g of Lys per 100 g of feed in four successive feeding periods, according to the NRC (1994) requirements. In the diets with a high level of Lys (L_H), the content of Lys was increased by 10% relative to the L_L diet. The two Arg levels in the experimental diets were determined so as to provide 95% and 105% Arg relative to the content of dietary Lys (A_L and A_H , respectively). As a result, the experiment compared the effects of using 4 experimental mixtures, with 2 levels of Lys and 2 levels of Arg ($L_L A_L$, $L_L A_H$, $L_H A_L$, $L_H A_H$). The same (higher than recommended by NRC, 1994) Met level was assumed in the experimental mixes and DL-Methionine was added to give 0.62, 0.59, 0.51 and 0.39 g of

Met per 100 g of feed in four successive feeding periods.

In accordance with a previously verified procedure, a basal feed was prepared for each of four 4-week feeding periods (Table 1), and its amino acid content was determined analytically. Then a portion of the basal feed was mixed with the appropriate amount of crystalline amino acids, i.e. L-lysine HCL, L-arginine HCL (Ajinomoto Eurolysine S.A.S, Amiens, France, 780 g lysine/kg and 990 g arginine/kg), and DL-methionine (MetA-MINO®, Evonik Degussa GmbH, Essen, Germany, 990 g methionine/kg). Both the basal and experimental feeds were produced in a local feed mill under the direct supervision of our representative. Ingredient composition and nutrient content of basal diets and the amounts of amino acids added to basal diets were presented in Ognik et al. (2021 b). During the experiment all birds had unlimited access to water and pelleted feed.

Growth trial and sample collection

Table 1. Ingredient composition and nutrient content of basal diets (g/100 g, as-fed basis) fed to turkeys

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
Ingredients				
Wheat	56.454	56.576	64.037	64.472
Maize	–	–	–	10.000
Soybean meal (48% CP)	25.380	27.722	21.121	9.324
Rapeseed meal	4.566	5.000	6.000	7.000
Potato protein	5.000	–	–	–
Soybean oil	0.892	3.088	4.725	4.371
Maize gluten meal	3.000	2.769	–	2.000
Sodium bicarbonate	0.200	0.20	0.100	0.200
Sodium chloride	0.158	0.161	0.217	0.095
Limestone	2.046	1.766	1.391	1.075
Monocalcium phosphate	1.707	1.390	1.409	0.558
L Lysine HCl	–	0.317	0.347	0.300
DL Methionine	0.195	0.231	0.163	0.072
L-Threonine	0.052	0.131	0.139	0.182
Choline chloride	0.10	0.100	0.100	0.100
Vitamin-mineral premix ¹	0.25	0.250	0.250	0.250
Titanium oxide	–	0.300	–	–
Calculated nutrient content²				
Metabolizable energy (kcal/kg)	2800	2900	3000	3150
Crude protein	26.35 (26.79)	24.0 (24.33)	20.3 (20.31)	17.0 (17.35)
Arginine	1.52 (1.50)	1.42 (1.38)	1.21 (1.25)	0.92 (0.89)
Lysine	1.30 (1.23)	1.35 (1.36)	1.20 (1.18)	0.90 (0.76)
Methionine	0.62 (0.61)	0.59 (0.56)	0.46 (0.45)	0.35 (0.38)
Methionine + Cysteine	1.09 (1.06)	1.03 (0.97)	0.85 (0.83)	0.71 (0.72)
Threonine	1.05 (1.03)	0.97 (0.94)	0.84 (0.83)	0.76 (0.78)
Calcium	1.25	1.10	0.95	0.65
Available phosphorus	0.65	0.55	0.47	0.32

¹Provided per kg diet (feeding periods: weeks 0–4, 5–8, 9–12 and 13–16): mg: retinol 3.78, 3.38, 2.88, and 2.52; cholecalciferol 0.13, 0.12, 0.10, and 0.09; a-tocopheryl acetate 100, 90, 80, and 70; vit. K₃ 5.8, 5.6, 4.8, and 4.2; thiamine 5.4, 4.7, 4.0, and 3.5; riboflavin 8.4, 7.5, 6.4, and 5.6; pyridoxine 6.4, 5.6, 4.8, and 4.2; cobalamin 0.032, 0.028, 0.024, and 0.021; biotin 0.32, 0.28, 0.24, and 0.21; pantothenic acid 28, 24, 20, and 18; nicotinic acid 84, 75, 64, and 56; folic acid 3.2, 2.8, 2.4, and 2.1; Fe 64, 60, 56 and 48; Mn 120, 112, 96, and 84; Zn 110, 103, 88, and 77; Cu 23, 19, 16, and 14; I 3.2, 2.8, 2.4, and 2.1; Se 0.30, 0.28, 0.24, and 0.21, respectively.

²The value in parentheses was determined analytically.

The body weight (BW) of birds was recorded and calculated on a pen basis. The feed conversion ratio (FCR) for the experimental period was calculated on a pen basis from body weight gain (BWG) and feed consumption, as kg of feed/kg of BWG. Mortality rates and causes were recorded daily, and the weights of dead birds were used to adjust the average FCR.

Blood samples were collected at 9 and 16 weeks of age from the wing vein of live birds. Blood was collected from 8 birds in each group (1 bird per replicate) with BW similar to the treatment average. Immediately after collection, blood samples were aliquoted into test tubes containing heparin as an anticoagulant. The samples were centrifuged for 15 min at 380 g and 4°C, and the resulting plasma was stored at -20°C until analysis.

At 6 and 16 weeks of age turkeys were killed at a slaughterhouse. The birds (without being transported) were electrically stunned (400 mA, 350 Hz), hung on a shackle line, and exsanguinated by a unilateral neck cut severing the right carotid artery and jugular vein. After a 3 min bleeding period, the birds were scalded at 61°C for 60 s, defeathered in a rotary drum picker for 25 s, and manually eviscerated. Liver samples were collected from 8 birds in each group for analysis of expression of selected genes at 6 week of age. In turn, at 16 week of age from 8 birds in each group the samples of liver and breast muscle were collected for redox status analyses.

Laboratory analyses

Laboratory analysis of the blood

The plasma content of total cholesterol (TC), triacylglycerols (TG), uric acid (UA), urea (UREA), total protein (TP), glucose (GLU) and creatinine (CREAT), bilirubin (BIL), albumin (ALB), as well as the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured using an automatic biochemical analyser (Plasma Diagnostic Instruments Horiba, Kyoto, Japan). Content of minerals (Ca, Mg, P, Fe, Cu, and Zn) in the blood samples was determined by FAAS (flame atomic absorption spectrometry). The content of thyroxine (T4) in the blood plasma was determined using kits produced by Cell Biolabs, Inc. (San Diego, USA). The concentration of malondialdehyde (MDA) in the blood of turkeys was determined using kits produced by Cell Biolabs, Inc. (San Diego, USA). The activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the blood of turkeys was determined by spectrometry using Ransel and Ransod diagnostic kits manufactured by Randox (Poland). Activity of catalase (CAT), as well as content of reduced glutathione (GSH) were determined in blood plasma by the method described by Ognik and Wiertelcki (2012).

Laboratory analysis of the breast muscle and liver

Activity of superoxide dismutase (SOD) and catalase (CAT), and the concentration of malondialdehyde

(MDA) and reduced glutathione (GSH) were determined in blood plasma by the method described by Ognik and Wiertelcki (2012).

Laboratory analysis of the liver

The relative mRNA expression levels of genes including catalase (CAT), superoxide dismutase 1 (SOD1) and glutathione peroxidase 1 (GPXI) were quantified in the liver. The protocol of qRT PCR analysis was adopted from Ognik et al. (2020). Collected samples of liver were immediately frozen in liquid nitrogen. Subsequently, RNA was isolated using the GeneMATRIX Universal RNA Purification Kit (Eurx, Gdańsk, Poland) following instructions provided by the manufacturer. The obtained RNA concentration was measured on a NanoDrop spectrophotometer (Nanodrop, NanoDrop Technologies, Wilmington, DE), and RNA integrity was verified on agarose gel under denaturing conditions. The qPCR reactions were performed on a LightCycler 480 II apparatus (Roche Applied Science, CA, USA) using SG qPCR Master Mix (Eurx, Gdańsk, Poland). The following conditions for the reaction were applied: (I) initial denaturation (one cycle at 95°C for 10 min) and (II) amplification (35 cycles of denaturation at 95°C for 10 s, primer annealing at 58°C for 10 s and DNA synthesis at 72°C for 20 s). β -Actin (*ACTB*) and Vimentin (*VIM*) genes were used as the endogenous control genes to normalize gene expression. The primers of investigated and reference genes used in this study were shown in Table 2.

Table 2. Genes and primers used in the study

Gene	Primer	Sequence (5'-3')	Melting temperature (°C)	Product size (nt)
<i>ACTB</i>	Forward	TACCCCAT-TGAACACGGCAT	58	96
	Reverse	CTCCTCAGGGGC-TACTCTCA		
<i>VIM</i>	Forward	GGAACAATGATGC-CCTGC	58	145
	Reverse	GCAAAATTCTCTC-CATTTCAC		
<i>SOD1</i>	Forward	TCTCATTACTACTCT-GCGTTCTT	58	199
	Reverse	CTGCCAACCATCT-TCCATTAC		
<i>CAT</i>	Forward	TGGTGACTATC-CTCTCATTC	58	83
	Reverse	GCCATCTGTCTAC-CTCTGT		
<i>GPXI</i>	Forward	GGCTTCAAACC-CAACTTC	58	151
	Reverse	GCGACCAGATGATG-TACT		

ACTB, β -actin; *VIM*, vimentin; *SOD1*, superoxide dismutase 1; *CAT*, catalase; *GPXI*, glutathione peroxidase 1.

Table 3. Blood biochemical parameters of turkeys (9 weeks of age)

Group (n=8) ¹	T4 ng/mL	AST U/L	ALT U/L	LDH U/L	ALP U/L	BIL µmol/l	TP g/l	ALB g/l	CREAT µmol/L	UREA mmol/L	UA mmol/L	TC mmol/L	TG mmol/L
L _L A _L	20.23	1777	1541	1778	1814	38.41	20.73	16.41 ^b	19.88	4.176	0.366 ^a	1.470	1.182
L _L A _H	23.45	1912	1628	1619	1831	31.11	18.68	13.87 ^b	21.75	3.565	0.249 ^b	1.573	1.334
L _H A _L	22.06	1658	1425	1588	1884	31.11	19.74	15.31 ^b	22.88	2.562	0.422 ^a	1.667	1.011
L _H A _H	19.14	1585	1395	1687	1848	35.48	15.19	22.15 ^a	24.38	2.597	0.450 ^a	1.463	0.987
SEM	1.120	35.92	27.26	53.51	27.28	1.525	0.985	0.650	1.142	0.225	0.019	0.062	0.067
Lysine (L)													
Low (L)	21.84	1844 a	1585 a	1699	1822	34.76	19.70	15.14	20.81	3.871	0.308	1.522	1.258
High (H)	20.60	1621 b	1410 b	1638	1866	33.30	17.47	18.73	23.63	2.580	0.436	1.565	0.999
Arginine (A)													
Low (L)	21.14	1717	1483	1683	1849	34.76	20.23	15.86	21.38	3.369	0.394	1.569	1.097
High (H)	21.30	1748	1511	1653	1839	33.30	16.94	18.01	23.06	3.081	0.350	1.518	1.161
P-value													
L	0.590	0.001	0.001	0.581	0.441	0.629	0.252	<0.001	0.236	0.003	<0.001	0.733	0.058
A	0.945	0.608	0.539	0.786	0.870	0.629	0.096	0.004	0.473	0.476	0.113	0.692	0.629
L×A	0.188	0.089	0.205	0.249	0.644	0.061	0.519	<0.001	0.936	0.424	0.013	0.233	0.506

a, b – values in same column with no common letters denote a significant difference (P≤0.05).

T4, thyroxine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; BIL, bilirubin; TP, total protein; ALB, albumin; CREAT, creatinine; UREA, urea; UA, uric acid; TC, total cholesterol; TG, triacylglycerols

¹ L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 4. Blood biochemical parameters of turkeys (16 weeks of age)

Group (n=8) ¹	T4 ng/mL	AST U/L	ALT U/L	LDH U/L	ALP U/L	BIL μmol/l	TP g/l	ALB g/l	CREAT μmol/L	UREA mmol/L	UA mmol/L	TC mmol/L	TG mmol/L
L _L A _L	19.87	1878	1630	1888	2328	65.77	28.50	14.49	20.63	1.884 b	0.469 b	1.828	1.952 b
L _L A _H	17.48	1709	1509	1994	2157	77.57	28.49	16.36	30.00	2.725 ^{ab}	0.659 a	2.162	2.596 a
L _H A _L	18.92	1975	1795	1863	2409	83.35	24.00	18.51	22.50	3.234 a	0.792 a	2.027	2.411 a
L _H A _H	16.98	1766	1511	1942	2361	75.61	23.76	23.23	29.25	2.470 ab	0.731 a	2.344	2.531 a
SEM	0.870	36.63	38.75	70.66	46.38	3.532	0.714	0.748	1.406	0.191	0.030	0.058	0.072
Lysine (L)													
Low (L)	18.68	1793	1570	1941	2242	71.67	28.49 a	15.43 b	25.31	2.305	0.564	1.995	2.274
High (H)	17.95	1871	1653	1903	2385	79.48	23.88 b	20.87 a	25.88	2.852	0.762	2.186	2.471
Arginine (A)													
Low (L)	19.39	1927 a	1712 a	1876	2369	74.56	26.25	16.50 b	21.56	2.559	0.631	1.928 ^b	2.181
High (H)	17.23	1737 b	1510 b	1968	2259	76.59	26.13	19.80 a	29.63	2.597	0.695	2.253 a	2.563
P-value													
L	0.686	0.255	0.234	0.797	0.128	0.276	0.001	<0.001	0.825	0.136	<0.001	0.061	0.107
A	0.234	0.008	0.006	0.534	0.236	0.776	0.919	0.002	0.003	0.916	0.152	0.002	0.003
L×A	0.899	0.762	0.247	0.928	0.500	0.176	0.927	0.157	0.607	0.033	0.008	0.929	0.035

a, b – values in same column with no common letters denote a significant difference (P≤0.05).

T4, thyroxine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; BIL, bilirubin; TP, total protein; ALB, albumin; CREAT, creatinine; UREA, urea; UA, uric acid; TC, total cholesterol; TG, triacylglycerols

¹L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with high lysine and low arginine levels; L_HA_L – diet with high lysine and high arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 5. Mineral content in turkeys' blood (9 weeks of age)

	9th week of age						16th week of age					
	Ca mmol/L	Mg mmol/L	P mmol/L	Zn μmol/L	Cu μmol/L	Fe μmol/L	Ca mmol/L	Mg mmol/L	P mmol/L	Zn μmol/L	Cu μmol/L	Fe μmol/L
Group (n=8) ¹												
L _L A _L	2.229 a	1.846	0.972 a	43.74	6.769	9.947	2.378	1.277	0.847 ab	41.61	6.849	11.07
L _L A _H	2.029 b	1.772	0.722 b	46.32	6.608	11.06	2.501	1.214	0.975 a	46.09	5.607	10.27
L _H A _L	2.158 ab	1.661	0.776 ab	46.13	9.332	8.626	2.242	1.127	0.959 a	48.96	8.611	12.19
L _H A _H	2.190 ab	1.822	0.871 ab	49.59	9.332	9.102	2.484	1.228	0.795 b	49.35	8.427	13.73
SEM	0.026	0.037	0.033	0.817	0.310	0.735	0.033	0.025	0.026	0.886	0.351	0.732
Lysine (L)												
Low (L)	2.129	1.809	0.847	45.03	6.689 b	10.50	2.440	1.246	0.911	43.85b	6.228b	10.67
High (H)	2.174	1.741	0.824	47.86	9.332 a	8.864	2.363	1.178	0.877	49.16a	8.519a	12.96
Arginine (A)												
Low (L)	2.194	1.753	0.874	44.94	8.050	9.287	2.310b	1.202	0.903	45.29	7.730	11.63
High (H)	2.110	1.797	0.796	47.95	7.970	10.08	2.493a	1.221	0.885	47.72	7.017	12.00
P value												
L	0.345	0.363	0.691	0.075	<0.001	0.285	0.201	0.164	0.479	0.001	<0.001	0.129
A	0.087	0.553	0.194	0.059	0.850	0.602	0.004	0.691	0.700	0.105	0.226	0.802
L×A	0.021	0.120	0.006	0.776	0.850	0.834	0.322	0.095	0.005	0.171	0.366	0.429

a, b – values in same column with no common letters denote a significant difference (P≤0.05).

¹L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Statistical analysis

The data were analysed by two way analysis of variance (ANOVA) using Statistica ver. 13 (StatSoft Inc. 2013). Significance of differences was verified by the Tukey test or the non-parametric Kruskal-Wallis test. Variation in the data was expressed as the standard error of the mean (SEM), and a value of $P < 0.05$ was considered statistically significant.

Results

Effect on the growth performance of turkeys

Body weights (BW), the feed conversion ratio (FCR) and mortality of turkeys were presented in Ognik et al. (2021 b). Considering that although not directly dealing with main results, the performance parameters are presented as additional practical information. Otherwise, the clarity of both publications would be reduced. Neither the 10% increase in Lys content relative to NRC (1994) recommendations nor the use of a diet containing a higher Arg level relative to Lys (105% Lys) had any effect on final BW and FCR, as well as on the mortality in the experimental turkeys (Ognik et al., 2021 b).

Effect on the metabolic parameters of turkeys

The use of Lys at a level 10% higher than NRC (1994) recommendations in the diet of turkeys resulted in a decrease in the activity of AST ($P=0.001$) and ALT ($P=0.001$) and in the content of UREA ($P=0.003$) in the plasma of the turkeys at 9 weeks of age (Table 3). The use of a higher Lys level in the diet also reduced the content of TP ($P=0.001$) and increased that of ALB ($P<0.001$) in the plasma of turkeys at 16 weeks (Table 4). The 10% increase in Lys relative to NRC (1994) recommendations caused an increase in the plasma content of Cu at both 9 and 16 weeks ($P<0.001$, both; Table 5). Furthermore, increased Lys content in the diet of 16-week-old turkeys increased Zn content ($P=0.001$) in the plasma (Table 5). The diet with a higher Arg level relative to Lys (105% Lys) reduced the activity of AST ($P=0.008$) and ALT ($P=0.006$) and increased the content of TC ($P=0.002$), CREAT ($P=0.003$) and ALB ($P=0.002$) in the plasma of 16-week-old turkeys (Table 4).

Two-way ANOVA showed Lys $\times$ Arg interactions in the case of ALB, UA, Ca and P content in the plasma of 9-week-old turkeys ($P<0.001$, $P=0.013$, $P=0.021$, and $P=0.006$, respectively) and in the case of UA, UREA, TG and P in the plasma of 16-week-old turkeys ($P=0.008$, $P=0.033$, $P=0.035$, and $P=0.005$) (Tables 3, 4, 5.). The significant Lys $\times$ Arg interactions resulted from the fact

that these parameters were influenced by both the Lys level and the Arg level, but their effect on the same parameter was often different, as confirmed by one-way analysis of variance. The results of one-way ANOVA showed that the Lys $\times$ Arg interaction for the content of UA, Ca and P in 9-week-old turkeys resulted from the fact that at the lower Lys level in the diet (in accordance with NRC (1994) recommendations), the use of a higher Arg level (105% Lys) reduced the plasma content of UA, Ca and P, which was not observed in the case of the 10% higher Lys level than that recommended by NRC (1994) (Tables 3 and 5). The Lys $\times$ Arg interaction for UA content in 16-week-old turkeys resulted from the fact that at the lower Lys level (in accordance with NRC (1994) recommendations), the use of the higher Arg level (105% Lys) caused an increase in plasma content of UA, which was not observed in the case of the Lys level 10% higher than that recommended by the NRC (1994) (Table 4). In the case of ALB and P in 16-week-old turkeys, the Lys $\times$ Arg interaction resulted from the fact that in the case of the Lys level 10% higher than that recommended by NRC (1994), a simultaneous increase in the Arg level (105% Lys) caused an increase in ALB and a decrease in P, which was not observed in the case of the diet containing a lower level of Lys (in accordance with NRC (1994) recommendations) (Tables 4 and 5). The interaction noted for UREA resulted from the fact that it was increased by the use of the 10% higher Lys level accompanying the use of a low level of Arg (95% Lys), which was not noted in the case of the lower level of Lys (in accordance with NRC (1994) recommendations) (Table 4). The interaction noted for TG resulted from the fact that the use of the higher level of Arg (105% Lys) and a level of Lys in accordance with NRC (1994) recommendations resulted in an increase in the plasma content of TG, which was not noted following the use of the higher level of Lys (Table 4).

Effect on the parameters of redox status and expression of genes responsible for regulation of redox reactions

In comparison with the lower level of Arg (95% Lys), the use of a higher level of this amino acid (105% Lys) in the diet of turkeys increased expression of the CAT gene ($P=0.048$) in the liver (Table 6). Increasing the lysine level by 10% relative to NRC (1994) recommendations and increasing Arg (105% Lys) in the diet caused an increase in the content of GSH ($P=0.042$ and $P=0.012$) in the plasma of 16-week-old turkeys (Table 7). In addition, higher CAT activity ($P=0.025$) and higher GSH content ($P=0.039$) were noted in the liver of 16-week-old turkeys receiving a diet with a higher Arg level (Table 8).

Table 6. Level of mRNA expression of selected genes in the liver of turkeys at 6 weeks of age

Group (n=8) ¹	<i>SOD1</i>	<i>CAT</i>	<i>GPXI</i>
L _L A _L	2.615	1.944	1.682
L _L A _H	3.955	3.554	1.655
L _H A _L	3.612	2.525	1.985
L _H A _H	3.155	2.645	2.758
SEM	0.234	0.223	0.229
Lysine (L)			
Low (L)	3.285	2.749	1.669
High (H)	3.383	2.585	2.371
Arginine (A)			
Low (L)	3.113	2.235 b	1.834
High (H)	3.555	3.100 a	2.206
P-value			
L	0.830	0.696	0.132
A	0.339	0.048	0.417
L×A	0.058	0.085	0.384

SOD1, superoxide dismutase 1; *CAT*, catalase; *GPXI*, glutathione peroxidase 1.

a, b – values in same column with no common letters denote a significant difference ($P \leq 0.05$).

¹L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 7. Redox parameters in turkeys' blood

	9th week of life					16th week of life				
	SOD U/gHb	CAT U/mL	GPX U/gHb	GSH ng/ml	MDA μmol/ml	SOD U/gHb	CAT U/mL	GPX U/gHb	GSH ng/ml	MDA μmol/ml
Group (n=8) ¹										
L _L A _L	1258.3	18.51	20.569	12.547	0.982	13125.2	35.62	18.11	11.236	1.025
L _L A _H	1277.6	17.18	21.069	12.621	0.987	13102.6	30.30	18.92	11.698	1.236
L _H A _L	1289.6	16.08	21.069	11.987	1.025	12847.5	28.92	17.69	12.365	1.098
L _H A _H	1148.5	15.63	20.887	12.069	1.007	13154.3	31.84	18.72	13.625	1.124
SEM	70.12	0.924	0.435	0.426	0.015	45.21	1.431	0.364	0.352	0.013
Lysine (L)										
Low (L)	1267.95	17.84	20.819	12.584	0.9845	13113.9	32.96	18.51	11.467	1.130
High (H)	1219.05	15.86	20.978	12.028	1.016	13000.9	30.38	18.21	12.995	1.111
Arginine (A)										
Low (L)	1273.9	17.30	20.81	12.584	1.0035	12986.3	32.27	17.90	11.80	1.061
High (H)	1183.7	16.40	20.93	12.028	1.0115	13077.6	31.07	18.46	13.31	1.117
P-value										
L	0.089	0.305	0.225	0.415	0.265	0.089	0.375	0.758	0.041	0.258
A	0.067	0.642	0.067	0.124	0.455	0.128	0.677	0.633	0.012	0.361
L×A	0.165	0.819	0.312	0.326	0.098	0.758	0.161	0.428	0.062	0.977

SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde.

¹L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 8. Redox parameters in selected tissues of turkeys (16 weeks of age)

	Breast muscle				Liver			
	MDA μmol/kg	SOD U/g protein	CAT U/g protein	GSH μmol/kg	MDA μmol/kg	SOD U/g protein	CAT U/g protein	GSH μmol/kg
Group (n=8) ¹								
L _L A _L	1.950	11.21	895.6	6.971	2.25	10.56	988.46	26.64
L _L A _H	1.964	11.36	961.4	6.945	2.31	10.31	1124.6	29.97
L _H A _L	2.031	11.45	958.4	7.047	2.17	10.66	1063.6	27.69
L _H A _H	2.014	12.24	997.8	7.121	2.29	11.08	1239.6	30.12
SEM	0.026	0.32	22.36	0.75	0.025	0.245	31.52	0.524
Lysine (L)								
Low (L)	1.957	11.285	928.5	6.958	2.28	10.43	1056.5	28.30
High (H)	2.022	11.845	978.1	7.084	2.23	10.87	1151.6	28.90
Arginine (A)								
Low (L)	1.990	11.33	927.0	7.009	2.21	10.61	1026.0	27.16
High (H)	2.018	12.04	987.9	7.102	2.26	10.97	1195.6	29.51
P-value								
L	0.174	0.785	0.062	0.645	0.178	0.547	0.059	0.245
A	0.325	0.321	0.214	0.355	0.233	0.631	0.025	0.039
L×A	0.077	0.087	0.645	0.124	0.097	0.089	0.364	0.625

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione.

¹L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Discussion

Many studies on broiler chickens have shown that increasing the Lys level relative to that recommended by the NRC (1994) improves growth performance (Nasr and Kheiri, 2011; Ojediran et al., 2018; Zarghi et al., 2020). Nasr and Kheiri (2011) added Lys in an amount greater than that recommended by the NRC (1994) to the 42-day diet of broiler chickens and noted an increase in their feed intake and final body weight. On the other hand, in our earlier studies, in which turkeys received a feed containing a high level of Lys similar to the recommendations of BUT (2013) and different levels of Arg (90, 100 and 110% Lys), no effect of the experimental treatments used on growth parameters such as BW and FCR was noted. This is consistent with the results of the studies presented in this study, in which turkeys received in their feed the Lys level increased by 10% in relation to the recommendations of the NRC (1994). Moreover, increasing the level of Lys by 10% in relation to the recommendations of the NRC (1994) in the feeding of turkeys did not affect the slaughter features such as the weight of offal (liver, gizzard and heart), muscles (breast, thigh and drumstick) and abdominal fat. The applied experimental treatment also had no effect on the pH and color of the breast muscle of the tested turkeys. (Konieczka et al., 2021).

The available literature proves that Lys plays an important role in the lipid metabolism in the liver, e.g. as a result of increasing the synthesis of L-carnitine, of which it is a precursor, enhancing the activity of coenzymes related to lipid metabolism and promoting β -oxidation of fatty acids. A deficiency of this amino acid in the diet can therefore lead to an increase in CHOL in the blood serum, excessive fat deposition in the body as well as liver damage (Ruan et al., 2019). In the present study, the use of a diet with Lys content 10% higher than recommended by the NRC (1994) had no effect on serum TC and TG levels but caused a decrease in AST and ALT activity in young turkeys. Because in physiological conditions these enzymes are located within cells, mainly hepatocytes, their activity in the blood plasma is relatively low. An increase in AST and ALT activity in the plasma is therefore regarded as a marker suggesting damage to liver cells (Kwo et al., 2017). In light of the above, the decrease noted in AST and ALT activity suggests that increasing the Lys level in the diet has a positive effect on lipid and protein metabolism in the liver. The available literature lacks similar studies on turkeys or other poultry species, but the results of studies on rats showed that the use of a diet with increased Lys (by 5%) reduced AST and ALT activity in the serum of these rodents (Wang et al., 1999). In the light of the above information, it can be assumed that the hepatoprotective effect of increased Lys levels in turkey nutrition, noted in our research, may be related to its participation in lipid metabolism (Lin et al., 2014; Ruan et al., 2019). It is likely that the adequate level of Lys supplied to the body with food will help to optimize

the metabolism of lipids, which do not accumulate in the liver and therefore do not damage the liver.

The available literature indicates that excess lysine in the diet can disrupt the urea cycle. This amino acid has a very strong affinity for arginase, which participates in the processes of ammonia detoxification. As a result of inhibiting the activity of this enzyme by lysine, harmful ammonia in the body may increase (Fico et al., 1982). The present study indicates that increasing the Lys level in the diet resulted in a decrease in the UREA level in the plasma of young turkeys. Similar results were obtained by Ishii et al. (2019) in a study on broiler chickens receiving a diet containing 50% more Lys than recommended by the NRC (1994). In birds, as uricotelic organisms, the main product of the metabolism of nitrogen compounds (proteins, amino acids and purines) is UA, not UREA (Rezende et al., 2017). It can be supposed, however, that the reduction in the UREA level as a result of increasing the amount of Lys in the diet may be caused by improved utilization of amino acids due to optimization of their intake. This seems to be confirmed in the present study by the increase in ALB content and accompanying decrease in TP content in the plasma of turkeys receiving Lys in an amount exceeding NRC (1994) recommendations by 10%. Jia et al. (2019) gave broiler chickens a diet containing 7% or 10% more Lys than the amount recommended by the NRC (1994) and noted an increase in both ALB and TP in the blood plasma. Hung et al. (2020) increased the lysine level in the diet of broilers from 0.1% to 0.3% and noted an increase in the plasma level of ALB. Albumins are synthesized in the liver and then secreted into the blood, which distributes them through the body. They perform numerous important functions, maintaining oncotic pressure, exhibiting pH buffering properties, and above all taking part in transport of substances in the body, including hormones, amino acids, medicines, and fatty acids (Miller and Jędrzejczak, 2001). Therefore their increased content in the body due to increased Lys intake by turkeys should be regarded as a beneficial effect. The decrease in TP in the serum of the birds was most likely due to increased synthesis of proteins and their increased level in the body of the turkeys, e.g. in the muscles, during rapid growth.

The results of our study indicate that a 10% increase in Lys in the diet relative to NRC (1994) recommendations resulted in an increase in the serum levels of Cu and Zn in young birds, whereas in older birds only the Cu level was increased. The available literature lacks information allowing us to compare our observations with those of other researchers and to explain the nature of these responses. There are, however, reports indicating that increased intake of Lys in the diet has a beneficial effect on Ca levels in the body by both improving the efficiency of intestinal absorption of this element and increasing its reabsorption in the kidneys (Civitelli et al., 1992). Although our study did not show a significant effect of the experimental treatment on the plasma level of Ca in the turkeys, it seems likely that Zn and Cu, as ele-

ments also present in divalent form, may be absorbed via similar mechanisms as Ca, thereby increasing their level in the plasma of turkeys.

Our results also showed that increasing Lys in the diet by 10% relative to NRC (1994) recommendations resulted in an increase in the plasma level of GSH in the turkeys. Although our study did not show a decrease in TC or TG in the plasma of turkeys receiving an increased amount of this amino acid in the diet, synthesis of L-carnitine from Lys can be presumed to lead to a decrease in lipid content in the body (especially polyunsaturated fatty acids susceptible to oxidation), and in consequence to a decrease in oxidation processes (Handique et al., 2019; Ghoreyshi et al., 2019). Moreover, L-carnitine regulates synthesis of NO, cellular respiration, and the activity of enzymes involved in antioxidant defence (Golzar Adabi et al., 2011). There are also reports that lysine and arginine compete with each other for the same cellular transport systems, so high levels of lysine in the diet may affect the degree of arginine utilization by the organism. Arginine is an amino acid necessary for the synthesis of NO, which in small amounts favorably stimulates the immune system, but at higher concentrations it can lead to undesirable free radical reactions enhancing the processes of oxidation and nitration of important cellular structures (Ognik et al., 2021 b). In the light of the results obtained in the course of our experiment, indicating an increase in GSH content with the simultaneous lack of deterioration in the activity of antioxidant enzymes and the intensification of lipid peroxidation both in plasma and in the examined turkey tissues, it can be assumed that the Lys: Arg ratio proposed by us is favorable and ensures the correct body antioxidant defence. This assumption seems to be additionally confirmed by our previous research in which it was proved that the combination of increased levels of Lys (+ 10%) and Arg (105% Lys) compared to the recommendations of the NRC (1994) lowered the level of protein nitration as well as protein and DNA oxidation (Ognik et al., 2021 b). The results of research by other authors confirm the beneficial effect of increased Arg intake in the diet of poultry on production parameters, most likely due to improved bioavailability of amino acids and increased synthesis of structural proteins, which can then be built into the muscles of the birds (Bulbul et al., 2013; Sirathonpong et al., 2019). The present study showed no significant effect of increased arginine in the diet relative to Lys (105%) on the growth performance of turkeys. There are reports indicating that the activity of certain liver enzymes involved in catabolism of amino acids is reduced by a low-protein diet and increases with intake of amino acids in the diet (Maroufyan et al., 2010). It is also presumed that increased intake of L-arginine results in overproduction of nitric oxide (NO), which functions as an intra- and intercellular transmitter, and thus can lead to liver cell damage. In consequence, the continuity of the cell membrane of hepatocytes is broken, and liver enzymes (e.g. AST and ALT) are released into the blood plasma, where

their activity increases significantly (Ozsoy et al., 2011). The results of the present study, however, did not confirm these reports; on the contrary, an increased level of Arg in the diet of turkeys relative to Lys (105% Lys) resulted in a decrease in AST and ALT activity. This indicates a beneficial effect of the Arg level used on the liver metabolism of turkeys. The available literature emphasizes the significant role of Arg in regulation of the lipid profile in poultry. Although the mechanisms determining it are still not well understood, an increase in the Arg level in the diet is known to decrease the level of TC in the plasma of birds (Ebrahimi et al., 2014; Yang et al., 2016). Ebrahimi et al. (2014) demonstrate that an increased Arg level in the diet of chickens positively affects the lipid profile and the amount of fat deposited in the body due to regulation of the expression of genes involved in lipid metabolism, such as acetyl-coenzyme A carboxylase (ACC), fatty acid synthase (FAS) and lipoprotein lipase (LPL). What is more, TC metabolism may be regulated by nitric oxide (NO), whose synthesis involves Arg. When NO is present in the body in physiological concentrations, it determines proper glucose uptake and oxidation as well as oxidation of fatty acids in insulin-sensitive tissues (the muscles, heart, liver and adipose tissue). In consequence, it can inhibit synthesis of glucose, glycogen and fat in target tissues (e.g. the liver and adipose tissue) and improve lipolysis in the adipocytes (Ognik et al., 2021 a). The results of our previous research indicated that an increased Arg level (110% Lys) in the diet of turkeys reduces the TC level in the serum while increasing the level of HDL (Ognik et al., 2021 a). In the light of results presented by other authors and our own previous research, it is difficult to explain the increase in TC in the present study in the plasma of turkeys fed a diet with increased Arg.

The results of our previous research (Ognik et al., 2021 a) showed that increasing the level of Arg (110%) relative to Lys (NRC, 1994) in the diet of turkeys resulted in a higher plasma level of CREAT. In the present study as well, increasing the level of Arg in the diet of turkeys to 105% Lys resulted in an increase in the plasma level of CREAT. Given that Arg and glycine are essential for creatinine synthesis, the increase in this parameter in the plasma of turkeys receiving increased Arg in the diet seems to be a consequence of this. The increased Arg level (105% Lys) in the diet of turkeys resulted in an increase in the content of ALB in the serum. This is consistent with results reported by Oso et al. (2017), who increased the addition of Arg in the diet of turkeys from 0 to 0.5 g/kg for an 8-week rearing period and noted a linear increase in the level of ALB in the serum. The increase in ALB in the blood plasma resulting from increased Arg in the diet is beneficial and indicative of efficient utilization of the amino acid.

The available literature indicates that the addition of Arg to the diet has a beneficial effect on antioxidant status (Atakisi et al., 2009). Arg is a precursor for nitric oxide synthesis catalysed by nitric oxide synthase (NOS). NO

produced in excessive amounts exerts an ad-verse effect on the body by increasing oxidation processes. Nevertheless, physiological values of this compound are essential for ensuring the normal functioning of the body. It performs an important function in immune processes and inhibits lipid peroxidation. Moreover, by inhibiting cytochrome oxidase activity, NO directly regulates oxidative phosphorylation in the mitochondria. What is more, physiological levels of NO exhibit antioxidant properties due to an increase in the intracellular concentration of reduced glutathione (Stępnik et al., 2001). Furthermore, it is presumed that the antioxidant properties of Arg may stem from the fact that by reducing the amount of fat in the body, it thereby limits lipid peroxidation processes (Fouad et al., 2013). In the present study, the use of a higher level of Arg (105% Lys) in the diet caused an increase in the plasma GSH level in older turkeys. In addition, higher GSH content was observed in the liver of the turkeys, as well as higher CAT activity and expression of the gene encoding it (CAT). The results of our study are in agreement with the literature reports cited and confirm that higher Arg levels in the diet have a beneficial effect on the body's antioxidant defence status.

Conclusions

An increase in the amount of Lys in the diet of turkeys by 10% relative to NRC nutritional recommendations (NRC, 1994) was not shown to improve growth performance, but had beneficial effects on the metabolism and antioxidant status of the birds. In comparison to the lower level of Arg (95% Lys) in the diet, an increase in the amount of this amino acid to 105% Lys did not improve growth performance, metabolism, or antioxidant status. An Arg level of 95% Lys can be used in a diet for turkeys containing 10% more Lys than the level recommended by the NRC (1994).

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