SELECTED METABOLIC, EPIGENETIC, NITRATION AND REDOX PARAMETERS IN TURKEYS FED DIETS WITH DIFFERENT LEVELS OF ARGININE AND METHIONINE

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Abstract

The amino acid guidelines formulated by British United Turkeys postulate higher levels of lysine (Lys) in turkey diets than those recommended by the National Research Council. However, any modifications in the Lys content of turkey diets should be accompanied by changes in the inclusion rates of other amino acids, including methionine (Met) and arginine (Arg). The research hypothesis postulates that the appropriate inclusion levels and ratios of arginine and methionine in turkey diets with high lysine content can improve the antioxidant status of turkeys without compromising their metabolism. The aim of this study was to determine the influence of different Arg and Met ratios in Lys-rich diets on biochemical indicators, redox status and epigenetic changes in turkeys. The turkeys were assigned to six groups with eight replicates per group and 18 birds per replicate. Six feeding programs, with three dietary Arg levels (90%, 100% and 110%) and two dietary Met levels (30% and 45%) relative to dietary Lys content were compared. During each of the four feeding phases, birds were fed *ad libitum* isocaloric diets with high Lys content. Our results show that in growing turkeys fed diets with high Lys content, the inclusion rate of Arg can be set at 90% of Lys content with no negative effects on their antioxidant status, metabolism or performance. Diets with high Arg content (110% Lys) are not recommended due to the risk of lipid and protein damage, and an undesirable increase in insulin and T4 levels. Regardless of dietary Arg levels, an increase in the Met inclusion rate from 30% to 45% of Lys content minimizes the oxidation of lipids, proteins and DNA, and increases the antioxidant defense potential of turkeys.

Key words: turkey, amino acid, biochemical parameter, hormone, blood

Diets with an appropriate amino acid profile play a key role in harnessing the genetic potential of fast-growing turkeys. The amino acid guidelines formulated by British United Turkeys (BUT, 2013) postulate higher levels of lysine (Lys) in turkey diets than those recommended by the National Research Council (NRC, 1994). However, any modifications in the Lys content of turkey diets should be accompanied by changes in the inclusion rates of other amino acids, including methionine (Met) and arginine (Arg), to maintain the “ideal” amino acid profile that promotes health, adequate growth performance and immunity in birds (Kidd et al., 1997; Zampiga et al., 2018).

Met is the first amino acid limiting the biological value of dietary protein in poultry. According to a review article by Jankowski et al. (2014), Met is essential for protein synthesis, and it plays various roles in the body. Met is responsible for cell division, and it is a precursor for the synthesis of carnitine and glutathione. Met is also a precursor of cysteine (Cys) which plays a key role in

maintaining antioxidant functions because it easily interacts with reactive oxygen species to produce methionine sulfoxides (Cudic et al., 2016). Many experiments conducted on chickens have demonstrated a close relationship between Arg and Met in promoting immune and antioxidant responses (Rama Rao et al., 2003; Tayade et al., 2006; Jahanian, 2009). Arg also limits the biological value of dietary proteins in poultry. It participates in numerous metabolic, immune and antioxidant processes (Fernandes and Murakami, 2010; Khajali and Wideman, 2010; Fouad et al., 2012). As a precursor of nitric oxide, creatine, ornithine, glutamate, polyamines, proline, glutamine, agmatine and dimethylarginine, Arg plays a major role in bird metabolism. Research has demonstrated that diets with a higher Arg content than that recommended by the NRC (1994) improve lipid metabolism and reduce abdominal fat in chickens (Le Mignon et al., 2009; Fouad et al., 2013) because Arg regulates the expression of genes responsible for fat metabolism. Other studies (Uni and Ferket, 2003; Oso et al., 2017) revealed that Arg im-

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proves the digestibility of carbohydrates and proteins by producing nitric oxide (NO) which stimulates the growth of microvessels in intestinal mucosa, improves morphology and absorption in the intestines. Turkeys lack a functional urea cycle (Tamir and Ratner, 1963) and are unable to synthesize endogenous Arg, which is why turkey diets are supplemented with exogenous Arg. According to Balnave and Brake (2002), both Arg deficiency and excess Arg supplementation can exert negative effects on amino acid concentrations in the blood plasma and muscles, which compromises bird growth. However, these effects are more pronounced at excess Lys (low Arg:Lys ratio) than at excess Arg (high Arg:Lys ratio). Broiler chicken diets containing excess Lys did not influence Arg digestibility and absorption, but it inhibited renal reabsorption of Arg and stimulated arginase activity in the kidneys (Balnave and Brake, 2002).

The amino acid requirements of turkeys formulated by the NRC (1994) and turkey breeding companies differ considerably. According to the NRC (1994), the inclusion level of Arg in turkey diets should reach 90–100% of Lys content, whereas higher Arg inclusion levels (102–105% Lys) are recommended by BUT (2013). The Met inclusion rate has been set at 30–38% relative to Lys content by the NRC (1994) and at 36–41% by BUT (2013). Our previous study of growing turkeys fed diets with low Lys content based on NRC (1994) guidelines demonstrated that antioxidant and immune defenses can be stimulated, and the oxidation and nitration of essential biological compounds can be limited when the Arg inclusion rate is set at 100% of Lys content, and the Met inclusion level reaches 45% of Lys content (Jankowski et al., 2020 a, b; Ognik et al., 2020 a). In a different study, the authors observed that in growing turkeys fed diets with high Lys content (close to BUT 2013 recommendations), Arg and Met inclusion rates can reach 90% and 45% of Lys content, respectively, without compromising the growth per-

formance or immune function of birds (Jankowski et al., 2020 c). However, little is known about the effects of different proportions of Arg and Met, relative to Lys content close to BUT (2013) recommendations on metabolism, antioxidant status, oxidation, nitration and epigenetic changes in turkeys.

The research hypothesis postulates that the appropriate inclusion levels and ratios of Arg and Met in turkey diets with high Lys content can improve the antioxidant status of turkeys without compromising their metabolism. The aim of this study was to determine the influence of different Arg and Met ratios in Lys-rich diets on biochemical indicators, redox status and epigenetic changes in turkeys.

Material and methods

Animals, housing and diets

A total of 864 one-day-old Hybrid Converter female turkey poults obtained from a commercial hatchery (Grelavi in Kętrzyn, NE Poland) were placed in pens on litter (wood shavings) and randomly allocated to six dietary treatments, with eight replicate pens (4 m² each; 2.0 m × 2.0 m) per treatment and 18 birds per pen. The stocking density in the initial stage of rearing was 4.5 birds/m². The initial body weight (BW) of one-day-old poults was 55.7±0.1 g. The temperature and lighting programs were consistent with the recommendations of British United Turkeys (2013). The protocol for the study was approved by the Local Ethics Committee (decision No. 82/2017), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU. Throughout the experiment, all birds had unlimited access to feed and water. The height of the watering and feeding lines was adapted to the growth stage of the birds.

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

Item	Feeding period (weeks)			
	1–4	5–8	9–12	13–16
1	2	3	4	5
Ingredients				
wheat	43.98	47.42	51.99	61.71
maize	10.00	10.00	10.00	10.00
soybean meal	28.77	26.54	23.85	15.24
rapeseed meal	3.00	3.00	3.00	3.00
potato protein	5.00	2.96	–	–
soybean oil	0.95	2.85	4.78	4.22
maize gluten meal	3.50	3.00	3.00	3.00
sodium bicarbonate	0.20	0.20	0.20	0.20
sodium chloride	0.15	0.16	0.16	0.12
limestone	2.07	1.87	1.64	1.45
monocalcium phosphate	1.94	1.55	0.96	0.65
L-threonine	0.09	0.10	0.07	0.06

Table 1 – contd.

1	2	3	4	5
choline chloride	0.10	0.10	0.10	0.10
vitamin-mineral premix ¹	0.25	0.25	0.25	0.25
titanium oxide	–	0.30	–	–
Calculated nutrient content (%)				
metabolizable energy (kcal/kg)	2820	2950	3100	3150
crude protein	27.0	24.5	21.5	18.5
arginine	1.58	1.44	1.27	1.04
lysine	1.36	1.19	0.97	0.76
methionine	0.44	0.39	0.34	0.30
met + cys	0.91	0.83	0.74	0.67
threonine	1.02	1.01	0.83	0.70
calcium	1.30	1.15	0.95	0.80
available phosphorus	0.70	0.60	0.47	0.40

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; α -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, 48 and 42; 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.

The birds were fed *ad libitum* isocaloric diets with high Lys content, approximately 1.83%, 1.67%, 1.48% and 1.20% in four successive feeding periods. The experiment had a completely randomized 3 × 2 factorial design with three levels of Arg (90%, 100% and 110%) and two levels of Met (30% and 45%), relative to the content of dietary Lys. Treatment Arg₉₀Met₃₀ received 90% Arg and 30% Met relative to the content of dietary Lys; treatment Arg₉₀Met₄₅ received 90% Arg and 45% Met relative to the content of dietary Lys; treatment Arg₁₀₀Met₃₀ received 100% Arg and 30% Met relative to the content of dietary Lys; treatment Arg₁₀₀Met₄₅ received 100% Arg and 45% Met relative to the content of dietary Lys; treatment Arg₁₁₀Met₃₀ received 110% Arg and 30% Met relative to the content of dietary Lys; treatment Arg₁₁₀Met₄₅ received 110% Arg and 45% Met relative to the content of dietary Lys.

The experimental diets were produced in a local feed mill under the direct supervision of a representative of the Department of Poultry Science, University of Warmia and Mazury. According to the experimental procedure, basal diets without supplemental Lys, Met and Arg were prepared for each of the four feeding periods (Table 1). After the addition of supplemental Arg and Met, their concentrations in experimental diets were also close to the values adopted in the experimental design model. Starter diets (days 1 to 28) were offered as crumbles, whereas grower and finisher diets (days 29 to 112) were prepared as 3 mm pellets at 65°C for 45 sec. The experimental diets did not contain any feed additives.

Growth performance and sample collection

The BW of birds was recorded and calculated on a pen basis. The feed conversion ratio (FCR; kg of feed/

kg of body weight gain – BWG) for the experimental period was calculated on a pen basis from BWG and feed consumption. 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 16 weeks of age from the wing vein of live birds. Blood was collected from eight birds in each group (one 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 the end of the experiment, birds were weighed after 8-hour feed deprivation, and one bird from each replicate representing the group average BW was selected and euthanized after electrical stunning. Birds were then hung on a processing line, and were bled out for 3 min by a unilateral neck cut severing the right carotid artery and jugular vein. The intestinal walls, livers and breast muscles were collected for analysis and then stored at –80°C.

Laboratory analyses

Samples of basal and experimental diets were analyzed in duplicate for crude protein (CP, N × 6.25) using Association of Official Analytical Chemists methods (AOAC, 2005). The amino acid analysis was performed according to Moore and Stein (1954). Liquid-phase hydrolysis of powdered samples was performed in 6M HCl containing 0.5% phenol at 110°C for 24 hours under an argon atmosphere. The hydrolyzates were lyophilized, dissolved in an appropriate volume of dilution buffer, and filtered through a 0.45 µm syringe filter before being applied to the amino acid analyzer. Sulfur-contain-

ing amino acids were analyzed as oxidation products obtained by performic acid oxidation (16 hours at 4°C) followed by standard hydrolysis with HCl. Amino acids were determined by ion-exchange chromatography with post-column derivatization with ninhydrin using an automatic amino acid analyzer according to the manufacturer's standard protocol, Ingos, Czech Republic (Davidson, 2003).

The plasma concentrations of total cholesterol (TC), triacylglycerols (TG), uric acid (UA), urea (UREA), glucose (GLU), bilirubin (BIL) and creatinine (CREAT), as well as the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) were measured using an automatic biochemical analyzer (Plasma Diagnostic Instruments Horiba, Kyoto, Japan). The concentrations of minerals (Ca, Mg, P, Fe, Cu, and Zn) in blood samples were determined by Flame Atomic Absorption Spectrometry (FAAS). The plasma levels of insulin, glucagon, thyroxine (T_4) and triiodothyronine (T_3) were determined with the use of kits produced by Cell Biolabs, Inc. (San Diego, USA). The plasma concentration of malondialdehyde (MDA) was determined with the use of 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). A diagnostic kit manufactured by Oxis International, Inc. (Portland, USA) was used to determine catalase (CAT) activity. The following indicators were also determined in the blood of turkeys: reduced glutathione (GSH) – by the Total Glutathione Assay (Cell Biolabs, Inc., San Diego, USA), and total antioxidant status (TAS) – with a Randox diagnostic kit (Poland). OxiSelect diagnostic kits (Cell Biolabs, Inc., San Diego, USA) were used to determine protein carbonyl (PC) derivatives as an indicator of the oxidation of amino acid residues, 3-nitrotyrosine (3-NT) as a marker of protein nitration, and 8-hydroxydeoxyguanosine (8-OHdG) as a marker of the oxidation of DNA bases. As described previously (Ognik and Wiertelicki, 2012), the following indicators of antioxidant status were determined in the intestinal wall, liver and breast muscles of turkeys: the activity of SOD and CAT, and the concentrations of MDA and total glutathione GSH+GSSG.

Statistical analysis

This experiment was performed in a completely randomized 3×2 factorial design, and the data (presented as the mean $\pm$ standard error of the mean) were subjected to two-way ANOVA to examine the effects of three levels of Arg (90%, 100% and 110%) and two levels of Met (30% and 45%). The Shapiro-Wilk and Levene tests were applied to test the model assumptions of normality and homogeneity of variance. When a significant interaction effect was noted (F test), treatment means were separated using the post hoc Tukey's test. The significance level

was set at $P = 0.05$, and statistical calculations were performed using the GLM procedures of the STATISTICA software system ver. 12.0 (StatSoft Inc., 2014).

Results

The growth performance

The applied dietary treatments had no effect on the BW of turkeys or FCR in any stage of the study (data presented by Jankowski et al., 2020 c) and throughout the experiment (Table 2), regardless of Arg and Met levels. In week 16, the average mortality rate was 1.43%, ranging from 0.7% in treatment Arg₉₀Met₄₅ to 2.0% in treatments Arg₉₀Met₃₀ and Arg₁₀₀Met₄₅.

Table 2. The performance (weeks 1–16 of age, $n = 8$) of turkeys fed diets with different levels of arginine and methionine

	BW 16 week (kg)	FCR (kg kg ⁻¹)	Mortality (%)
Treatment ¹			
Arg ₉₀ Met ₃₀	11.43	2.44	2.0
Arg ₉₀ Met ₄₅	11.45	2.44	0.7
Arg ₁₀₀ Met ₃₀	11.38	2.44	1.3
Arg ₁₀₀ Met ₄₅	11.43	2.44	2.0
Arg ₁₁₀ Met ₃₀	11.47	2.44	1.3
Arg ₁₁₀ Met ₄₅	11.49	2.42	1.3
SEM	0.031	0.011	–
Arg level (%)			
90	11.44	2.44	1.3
100	11.40	2.44	1.7
110	11.48	2.43	1.3
Met level (%)			
30	11.43	2.44	1.6
45	11.46	2.43	1.3
P-value			
Arg	0.610	0.947	–
Met	0.625	0.869	–
Arg $\times$ Met	0.988	0.876	–

¹Treatment: Arg₉₀Met₃₀ received 90% Arg and 30% Met relative to the content of dietary Lys; Arg₉₀Met₄₅ received 90% Arg and 45% Met relative to the content of dietary Lys; Arg₁₀₀Met₃₀ received 100% Arg and 30% Met relative to the content of dietary Lys; Arg₁₀₀Met₄₅ received 100% Arg and 45% Met relative to the content of dietary Lys; Arg₁₁₀Met₃₀ received 110% Arg and 30% Met relative to the content of dietary Lys; Arg₁₁₀Met₄₅ received 110% Arg level and 45% Met level relative to the content of dietary Lys. BW – body weight, FCR – feed conversion ratio.

The metabolic parameters

Arg₉₀ increased plasma TG concentrations ($P=0.042$), compared with the medium Arg content (100% Lys). Turkeys fed diets with the highest Arg content (110% Lys) had lower plasma urea concentrations ($P=0.008$) than those receiving diets with the medium Arg content (100% Lys). An increase in the inclusion rate of Met from

30% to 45% of Lys content increased ($P=0.044$) plasma TG concentrations (Table 3). The Arg $\times$ Met interaction effect was detected for plasma GLU concentrations ($P=0.036$): an increase in the inclusion rate of Met from 30% to 45% of Lys content led to an increase in plasma GLU levels at the lowest dietary Arg content (90% Lys), but not at the medium or highest Arg content (100% and 110% Lys, respectively) (Table 3). An Arg $\times$ Met interaction ($P=0.028$) was also noted for UA levels: an increase in the inclusion rate of Met from 30% to 45% of Lys content caused an increase in plasma UA levels at the lowest and medium dietary Arg content (90% and 100% Lys), but not at the highest Arg content (110% Lys). An Arg $\times$ Met interaction ($P=0.033$) was also observed for plasma BIL concentrations: an increase in the inclusion rate of Met from 30% to 45% of Lys content led to a decrease in plasma BIL levels at the lowest and medium dietary Arg content (90% and 100% Lys), but not at the highest Arg content (110% Lys). An Arg $\times$ Met interaction ($P=0.004$) was also found for plasma CREAT concentrations: an increase in the inclusion rate of Met from 30% to 45% of Lys content caused a decrease in plasma CREAT levels at the lowest and highest dietary Arg content (90% and 110% Lys), but not at the medium Arg content (100%

Lys) (Table 3). In comparison with the lowest and medium Arg content (90% and 100% Lys), the highest Arg content (110% Lys) enhanced AST activity in the blood plasma of turkeys ($P<0.001$) (Table 4). Plasma Mg levels were lower ($P<0.001$) in turkeys fed diets with the lowest Arg content (90% Lys) than in those receiving diets with higher Arg rates (100% and 110% Lys). An increase in the inclusion rate of Met from 30% to 45% of Lys content contributed to a decrease in plasma Mg levels ($P=0.027$). In comparison with diets with the medium Arg content (100% Lys), diets with the highest Arg content (110% Lys) tended to decrease plasma Zn levels ($P=0.055$) (Table 5).

In comparison with the lowest Arg content (90% Lys), the medium and highest Arg content (100% and 110% Lys) led to an increase in plasma insulin levels ($P<0.001$). In comparison with turkeys fed diets with the lowest and medium Arg content (90% and 100% Lys), those receiving diets with the highest Arg content (110% Lys) were characterized by lower glucagon levels ($P<0.001$) and higher T4 levels ($P<0.001$) in the blood plasma. Met₄₅ resulted in a decrease in the plasma levels of insulin ($P=0.012$) and glucagon ($P=0.005$) (Table 6).

Table 3. Blood biochemical parameters in turkeys fed diets with different levels of arginine and methionine

Item	GLU (mmol L ⁻¹)	TC (mmol L ⁻¹)	TG (mmol L ⁻¹)	UREA (mmol L ⁻¹)	UA (mmol L ⁻¹)	BIL (μ mol L ⁻¹)	CREAT (μ mol L ⁻¹)
Treatment ¹							
Arg ₉₀ Met ₃₀	18.29 b	2.553	3.064	4.558	0.361 b	60.42 ab	28.13 a
Arg ₉₀ Met ₄₅	21.11 a	2.541	3.165	4.635	0.486 ab	44.28 b	19.50 b
Arg ₁₀₀ Met ₃₀	20.20 ab	2.478	2.092	5.449	0.419 ab	62.80 ab	20.29 b
Arg ₁₀₀ Met ₄₅	19.47 ab	2.986	2.587	5.653	0.444 ab	62.52 ab	28.13 a
Arg ₁₁₀ Met ₃₀	20.35 ab	3.232	2.439	4.787	0.509 a	55.06 ab	23.63 ab
Arg ₁₁₀ Met ₄₅	19.44 ab	2.745	3.408	3.743	0.409 ab	66.64 a	23.25 ab
SEM	0.329	0.097	0.136	0.182	0.017	2.241	1.012
Arg level (%)							
90	19.70	2.547	3.114 a	4.596 ab	0.423	52.35	23.81
100	19.83	2.732	2.339 b	5.551 a	0.432	62.66	24.21
110	19.90	2.989	2.923 ab	4.265 b	0.459	60.85	23.44
Met level (%)							
30	19.61	2.754	2.531 b	4.932	0.430	59.43	24.01
45	20.01	2.758	3.053 a	4.677	0.446	57.81	23.63
P-value							
Arg	0.967	0.168	0.042	0.008	0.651	0.111	0.945
Met	0.539	0.987	0.044	0.449	0.613	0.701	0.837
Arg $\times$ Met	0.036	0.109	0.379	0.254	0.028	0.033	0.004

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

¹Treatment: Arg₉₀Met₃₀ received 90% Arg and 30% Met relative to the content of dietary Lys; Arg₉₀Met₄₅ received 90% Arg and 45% Met relative to the content of dietary Lys; Arg₁₀₀Met₃₀ received 100% Arg and 30% Met relative to the content of dietary Lys; Arg₁₀₀Met₄₅ received 100% Arg and 45% Met relative to the content of dietary Lys; Arg₁₁₀Met₃₀ received 110% Arg and 30% Met relative to the content of dietary Lys; Arg₁₁₀Met₄₅ received 110% Arg level and 45% Met level relative to the content of dietary Lys. GLU – glucose, TC – total cholesterol, TG – triacylglycerols, UREA – urea, UA – uric acid, BIL – bilirubin, CREAT – creatinine.

Table 4. Blood enzyme activities in turkeys fed diets with different levels of arginine and methionine

	ALT (U L ⁻¹)	ALP (U L ⁻¹)	LDH (U L ⁻¹)	AST (U L ⁻¹)
Treatment ¹				
Arg ₉₀ Met ₃₀	7.013	1341.1	1887.9	238.7
Arg ₉₀ Met ₄₅	6.678	1396.6	1869.1	266.6
Arg ₁₀₀ Met ₃₀	6.444	1386.1	1861.9	251.6
Arg ₁₀₀ Met ₄₅	5.160	1256.0	1777.9	283.0
Arg ₁₁₀ Met ₃₀	6.193	1248.2	1706.8	382.9
Arg ₁₁₀ Met ₄₅	6.358	1149.2	1685.9	379.8
SEM	0.247	48.37	53.26	10.36
Arg level (%)				
90	6.846	1368.9	1878.5	252.7 b
100	5.802	1321.0	1819.9	267.3 b
110	6.276	1198.7	1696.4	381.3 a
Met level (%)				
30	6.550	1325.1	1818.9	291.1
45	6.066	1267.2	1777.6	309.8
P-value				
Arg	0.233	0.358	0.394	<0.001
Met	0.330	0.561	0.710	0.136
Arg × Met	0.480	0.715	0.963	0.458

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

¹Treatment: Arg90Met30 received 90% Arg and 30% Met relative to the content of dietary Lys; Arg90Met45 received 90% Arg and 45% Met relative to the content of dietary Lys; Arg100Met30 received 100% Arg and 30% Met relative to the content of dietary Lys; Arg100Met45 received 100% Arg and 45% Met relative to the content of dietary Lys; Arg110Met30 received 110% Arg and 30% Met relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys. ALT – alanine aminotransferase, ALP – alkaline phosphatase, LDH – lactate dehydrogenase, AST – aspartate aminotransferase.

Table 5. Blood mineral concentrations in turkeys fed diets with different levels of arginine and methionine

	Fe ($\mu\text{mol L}^{-1}$)	Cu ($\mu\text{mol L}^{-1}$)	Zn ($\mu\text{mol L}^{-1}$)	Ca (mmol L ⁻¹)	P (mmol L ⁻¹)	Mg (mmol L ⁻¹)
Treatment ¹						
Arg ₉₀ Met ₃₀	17.01	37.29	53.85	3.907	1.476	1.676
Arg ₉₀ Met ₄₅	19.76	35.97	50.03	3.986	1.607	1.370
Arg ₁₀₀ Met ₃₀	17.73	35.20	59.88	4.220	1.467	2.364
Arg ₁₀₀ Met ₄₅	19.54	36.61	54.41	4.240	1.649	2.212
Arg ₁₁₀ Met ₃₀	21.45	37.41	51.79	4.940	1.730	3.054
Arg ₁₁₀ Met ₄₅	16.59	37.77	48.08	4.405	1.714	2.544
SEM	0.830	0.320	1.277	0.140	0.041	0.105
Arg level (%)						
90	18.38	36.63	51.94 ab	3.947	1.541	1.523 c
100	18.64	35.91	57.14 a	4.230	1.558	2.288 b
110	19.02	37.59	49.93 b	4.672	1.722	2.799 a
Met level (%)						
30	18.73	36.63	55.17	4.356	1.557	2.365 a
45	18.63	36.78	50.84	4.210	1.657	2.042 b
P-value						
Arg	0.952	0.096	0.055	0.111	0.137	<0.001
Met	0.953	0.814	0.083	0.603	0.220	0.027
Arg × Met	0.141	0.205	0.947	0.611	0.579	0.589

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

¹Treatment: Arg90Met30 received 90% Arg and 30% Met relative to the content of dietary Lys; Arg90Met45 received 90% Arg and 45% Met relative to the content of dietary Lys; Arg100Met30 received 100% Arg and 30% Met relative to the content of dietary Lys; Arg100Met45 received 100% Arg and 45% Met relative to the content of dietary Lys; Arg110Met30 received 110% Arg and 30% Met relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Table 6. Blood hormone levels in turkeys fed diets with different levels of arginine and methionine

Item	Insulin (ng mL ⁻¹)	Glucagon (pg mL ⁻¹)	T ₃ (ng mL ⁻¹)	T ₄ (ng mL ⁻¹)
Treatment ¹				
Arg ₉₀ Met ₃₀	0.640	34.41	4.547	79.69
Arg ₉₀ Met ₄₅	0.602	31.06	4.499	78.79
Arg ₁₀₀ Met ₃₀	0.740	32.50	4.729	84.85
Arg ₁₀₀ Met ₄₅	0.708	30.32	4.590	83.31
Arg ₁₁₀ Met ₃₀	0.882	26.56	4.994	108.35
Arg ₁₁₀ Met ₄₅	0.822	26.02	4.751	98.14
SEM	0.016	0.544	0.089	1.988
Arg level (%)				
90	0.621 c	32.73 a	4.523	79.24 b
100	0.724 b	31.41 a	4.659	84.08 b
110	0.852 a	26.29 b	4.872	103.24 a
Met level (%)				
30	0.754 a	31.15 a	4.757	90.96
45	0.710 b	29.14 b	4.613	86.74
P-value				
Arg	<0.001	<0.001	0.298	<0.001
Met	0.012	0.005	0.434	0.108
Arg × Met	0.774	0.250	0.908	0.267

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

¹Treatment: Arg₉₀Met₃₀ received 90% Arg and 30% Met relative to the content of dietary Lys; Arg₉₀Met₄₅ received 90% Arg and 45% Met relative to the content of dietary Lys; Arg₁₀₀Met₃₀ received 100% Arg and 30% Met relative to the content of dietary Lys; Arg₁₀₀Met₄₅ received 100% Arg and 45% Met relative to the content of dietary Lys; Arg₁₁₀Met₃₀ received 110% Arg and 30% Met relative to the content of dietary Lys; Arg₁₁₀Met₄₅ received 110% Arg level and 45% Met level relative to the content of dietary Lys. T₃ – triiodothyronine, T₄ – thyroxine.

Table 7. Blood redox parameters of 16-week-old turkeys fed diets differing in arginine and methionine content (n = 8)

Item	MDA (μmol L ⁻¹)	PC (nmol mg ⁻¹)	3-NT (nmol L ⁻¹)	8-OHdG (ng mL ⁻¹)	SOD (U gHb ⁻¹)	GPx (U gHb ⁻¹)	CAT (U mL ⁻¹)	TAS (mmol L ⁻¹)	GSH+GSSG (μg mL ⁻¹)
Treatment ¹									
Arg ₉₀ Met ₃₀	0.908 b	4.088	143.0	8.298	1531.9	20.35	13.37	1.752	6.95
Arg ₉₀ Met ₄₅	1.037 a	3.977	136.2	7.952	1701.5	22.15	13.68	1.836	9.76
Arg ₁₀₀ Met ₃₀	1.009 ab	4.488	160.4	8.456	1207.9	21.21	18.16	1.670	13.56
Arg ₁₀₀ Met ₄₅	0.971 ab	3.824	157.2	7.907	1336.2	24.69	15.41	1.707	15.22
Arg ₁₁₀ Met ₃₀	1.044 a	4.771	184.7	8.728	732.4	22.53	13.53	1.691	13.50
Arg ₁₁₀ Met ₄₅	0.943 ab	4.465	173.6	8.004	828.2	23.58	16.08	1.765	17.74
SEM	0.016	0.078	2.944	0.077	70.78	0.437	0.659	0.030	0.791
Arg level (%)									
90	0.972	4.032 b	139.6 c	8.125	1616.7 a	21.25	13.52	1.794	8.35 b
100	0.990	4.156 b	158.8 b	8.182	1272.1 b	22.95	16.78	1.689	14.39 a
110	0.994	4.618 a	179.1 a	8.366	780.3 c	23.05	14.80	1.728	15.62 a
Met level (%)									
30	0.987	4.449 a	162.7	8.494 a	1157.4	21.36 b	15.02	1.704	11.34 b
45	0.984	4.089 b	155.7	7.954 b	1288.6	23.47 a	15.06	1.769	14.24 a
P-value									
Arg	0.835	0.002	<0.001	0.317	<0.001	0.139	0.127	0.379	<0.001
Met	0.921	0.008	0.054	<0.001	0.213	0.013	0.976	0.295	0.027
Arg × Met	0.013	0.223	0.660	0.522	0.959	0.467	0.253	0.948	0.709

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

¹Treatment: Arg₉₀Met₃₀ received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg₉₀Met₄₅ received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg₁₀₀Met₃₀ received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg₁₀₀Met₄₅ received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg₁₁₀Met₃₀ received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg₁₁₀Met₄₅ received 110% Arg level and 45% Met level relative to the content of dietary Lys; MDA – malondialdehyde, PC – protein carbonyl, 3-NT – 3-nitrotyrosine, 8-OHdG – 8-hydroxydeoxyguanosine.

SOD – superoxide dismutase, GPx – glutathione peroxidase, CAT – catalase, TAS – total antioxidant status, GSH+GSSG – total glutathione.

Table 8. Redox parameters of 16-week-old turkeys fed diets differing in arginine and methionine content (n = 8)

Item	Intestinal wall				Liver				Breast muscles			
	MDA ($\mu\text{mol kg}^{-1}$)	SOD (U g^{-1} protein)	CAT (U g^{-1} protein)	GSH+GSSG ($\mu\text{mol kg}^{-1}$)	MDA ($\mu\text{mol kg}^{-1}$)	SOD (U g^{-1} protein)	CAT (U g^{-1} protein)	GSH+GSSG ($\mu\text{mol kg}^{-1}$)	MDA ($\mu\text{mol kg}^{-1}$)	SOD ($\mu\text{mol kg}^{-1}$ protein)	CAT ($\mu\text{mol kg}^{-1}$ protein)	GSH+GSSG ($\mu\text{mol kg}^{-1}$)
Treatment¹												
Arg ₉₀ Met ₃₀	1.260	17.60	1451.8	5.604	2.258	8.435	1139.5	29.79	1.954	11.09	881.3	9.589
Arg ₉₀ Met ₄₅	1.307	16.51	1323.4	5.461	2.325	10.878	1568.2	29.86	1.613	11.39	737.3	8.310
Arg ₁₀₀ Met ₃₀	1.404	15.21	1571.9	5.790	3.720	7.358	1552.2	30.51	1.609	10.94	1288.5	5.602
Arg ₁₀₀ Met ₄₅	1.404	14.21	1433.4	4.774	2.676	8.149	1338.0	32.35	1.519	15.30	1301.8	5.367
Arg ₁₁₀ Met ₃₀	1.742	13.47	1189.0	4.840	3.339	8.011	1917.6	32.84	2.036	13.02	1301.4	5.549
Arg ₁₁₀ Met ₄₅	1.612	8.84	1009.7	4.329	2.848	8.614	1484.3	30.18	2.089	13.50	1265.1	6.341
SEM	0.083	0.983	61.71	0.195	0.165	0.597	91.72	1.066	0.104	0.674	44.30	0.366
Arg level (%)												
90	1.283	17.06 a	1387.6 ab	5.533	2.291 b	9.656	1353.9	29.82	1.783	11.24	809.3 b	8.950 a
100	1.404	14.71 ab	1502.7 a	5.282	3.198 a	7.753	1445.1	31.43	1.564	13.12	1295.2 a	5.485 b
110	1.677	11.15 b	1099.4 b	4.585	3.094 a	8.312	1701.0	31.51	2.063	13.26	1283.2 a	5.945 b
Met level (%)												
30	1.469	15.43	1404.2	5.411	3.106	7.935	1536.4	31.05	1.866	11.69	1157.1	6.914
45	1.441	13.19	1255.5	4.855	2.616	9.213	1463.5	30.80	1.740	13.40	1101.4	6.673
P-value												
Arg	0.156	0.047	0.022	0.120	0.041	0.429	0.273	0.784	0.159	0.396	<0.001	<0.001
Met	0.869	0.243	0.212	0.150	0.120	0.299	0.687	0.910	0.547	0.209	0.368	0.689
Arg × Met	0.905	0.672	0.983	0.645	0.347	0.795	0.139	0.709	0.738	0.386	0.566	0.377

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

¹Treatment: Arg90Met30 received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg₉₀Met₄₅ received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg₁₀₀Met₃₀ received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg₁₀₀Met₄₅ received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg₁₁₀Met₃₀ received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg₁₁₀Met₄₅ received 110% Arg level and 45% Met level relative to the content of dietary Lys; MDA – malondialdehyde, SOD – superoxide dismutase, CAT – catalase, GSH+GSSG – total glutathione.

The epigenetic, nitration and redox parameters

In comparison with turkeys fed diets with the lowest Arg content (90% Lys), those receiving diets with the medium and highest Arg content (100% and 110% Lys) were characterized by higher plasma levels of 3-NT and GSH (both $P<0.001$), and lower SOD activity ($P<0.001$) in erythrocytes. The highest dietary Arg content (110% Lys) caused an increase in plasma PC levels ($P=0.002$), compared with the medium and lowest Arg content (100% and 90% Lys). Regardless of dietary Arg levels, an increase in the inclusion rate of Met from 30% to 45% of Lys content contributed to a decrease in the plasma levels of PC ($P=0.008$), 3-NT ($P=0.054$) and 8-OHdG ($P<0.001$), and an increase in the activity of GPx ($P=0.013$) and GSH ($P=0.027$) (Table 7).

Diets with the highest Arg content (110% Lys) decreased the activity of SOD ($P=0.047$) and CAT ($P=0.022$) in the intestinal wall, but they had no effect on the activity of these enzymes in the liver. In comparison with the lowest Arg content (90% Lys), the medium and highest Arg content (100% and 110% Lys) led to an increase in MDA levels ($P=0.041$) in the liver, and to an increase in CAT activity ($P<0.001$) and a decrease in GSH concentrations ($P<0.001$) in the breast muscles of turkeys (Table 8).

Discussion

The presence of significant interactions between Arg and Met inclusion levels in turkey diets with high Lys could be attributed to the fact that selected indicators (GLU, UA, BIL, CREAT) were affected by both Arg and Met content, but in some cases, Arg and Met exerted a different influence on the same parameter.

Arg promotes growth performance in turkeys because it acts as a substrate for creatine biosynthesis (Khalifeh-Gholi and Jahanian, 2012; Jankowski et al., 2020 a; Ognik et al., 2020 a). Met is also directly implicated in creatine synthesis. Met donates a methyl group to glyco-cyaminate (the biological precursor for creatine synthesis in birds) which is synthesized from Arg and Gly. In the present study, differences in Arg and Met inclusion rates in diets, relative to Lys content (which was close to BUT 2013 recommendations, i.e. high) did not affect the final BW of turkeys. In our previous experiment where the Lys content of turkey diets was based on NRC (1994) guidelines, an increase in Arg and Met inclusion rates to 100% and 45% of Lys content, respectively, improved BWG (Jankowski et al., 2020 a).

Lys and Met stimulate pancreatic secretion of insulin into the blood stream (Handique et al., 2019 a, b). The presence of a relationship between glucagon and methionine may be attributed to glucagon's potent stimulatory effects on methionine uptake in the liver (Flakoll et al., 1994). In the current study, regardless of the dietary levels of Arg (90–100% Lys), an increase in the inclusion rate of Met from 30% to 45% of Lys content decreased

plasma insulin and glucagon concentrations. Numerous authors have demonstrated that Arg enhances insulin secretion. Hyperinsulinemia can also lead to hyperglycemia and the development of insulin resistance (Scherrer et al., 1994; Steinberg et al., 1994; van Loon et al., 2000). In our previous experiment where the Lys content of turkey diets was consistent with NRC (1994) recommendations, different inclusion levels of Arg (90–110% Lys) did not affect plasma insulin levels. However, diets with Lys content based on NRC (1994) guidelines and a low Arg level (90% Lys) contributed to an increase in glucagon concentrations (Ognik et al., 2020 a). In the present experiment, diets with high Lys content and an equivalent or higher Arg level (100% and 110% Lys) increased plasma insulin levels, but did not affect GLU concentrations. Plasma glucagon levels decreased in turkeys fed diets with the highest Arg content (110% Lys) although, as demonstrated by Takahashi and Akiba (1995), Arg also stimulates the secretion of glucagon, the most potent lipolytic hormone in poultry.

A review article by Jobgen et al. (2006) revealed that insulin and glucagon regulate TG lipolysis. When TG lipolysis is activated, hormone-sensitive lipase (HSL) and perilipins are phosphorylated by cAMP-dependent protein kinase, which stimulates TG lipolysis. Glucagon and catecholamines increase intracellular cAMP levels by activating adenyl cyclase, whereas insulin decreases intracellular concentrations of cAMP by stimulating the activity of phosphodiesterase 3B (Holm, 2003). In the present study, diets with a high content of Lys as well as Arg (100% and 110% Lys) decreased TG and glucagon levels and increased plasma insulin concentrations. These results suggest that TG lipolysis was probably regulated by endogenous NO, rather than by glucagon or insulin. Nitric oxide synthesized from Arg increases intracellular levels of cyclic guanosine monophosphate (cGMP) and suppresses the activity of phosphodiesterase 5 which hydrolyzes cAMP and cGMP (Tansey et al., 2004). However, excess NO may oxidize and inactivate catecholamines nonenzymatically, thus reducing the rate of stimulated lipolysis in adipocytes (Jobgen et al., 2006). According to the literature, dietary supplementation with Arg can augment the treatment of lipid metabolism disorders by suppressing NOS inhibitors and therefore lowering the plasma levels of TG, TC and LDL-TC (Wu et al., 2009; Fouad et al., 2013; Yang et al., 2016). The inclusion of L-Arg into the diets of broiler chickens at 250% of their requirements at 1 to 49 days of age considerably decreased serum TG levels (Emadi et al., 2011). Serum TG levels and abdominal fat deposition decreased in 42-day-old Japanese quails injected with 2% L-Arg on incubation day 0 (Al-Daraji et al., 2011). Goudarz et al. (2009) reported a decrease in TG and TC concentrations in the blood of rabbits fed Arg-supplemented diets. In our previous study of turkeys, different dietary inclusion rates of Arg (90–110% Lys) (NRC, 2014) did not affect TG levels, but the lowest Arg level (90% Lys) increased plasma TC concentrations (Ognik et al., 2020 a). In the

current experiment, diets with high Lys (BUT, 2013) and low Arg (90% Lys) content increased plasma TG levels. Regardless of the Arg:Lys ratio, an increase in the Met inclusion rate from 30% to 45% of Lys content increased plasma TG concentrations. Plasma TG levels also increased in a study of young turkeys fed diets with high Lys content and low levels of Arg (90% Lys) and Met (30% Lys) (Ognik et al., 2020 b). However, an increase in the dietary inclusion rate of Met from 30% to 45% of Lys content decreased plasma TG levels in turkeys fed diets with high Lys content and low Arg content (90% Lys) (Ognik et al., 2020 b). According to a review article by Oda (2006), sulfur-containing amino acids control lipid metabolism, and their adequate levels in the diet desirably decrease the plasma concentrations of TC and TG.

Arg is hydrolyzed by the enzyme arginase, which leads to the production of urea and ornithine. According to Ruiz-Feria et al. (2001), urea production increases with a rise in the Arg content of bird diets. Excess Arg has no significant effect on Lys metabolism, whereas excess Lys strongly antagonizes the metabolism of Arg (Ruiz-Feria et al., 2001). The antagonism between Arg and Lys significantly increases the activity of renal arginase, which induces the breakdown of Arg. In the present experiment, diets with a high content of Lys and Arg (110% Lys) decreased plasma urea levels, which is a surprising result. Decreased urea concentrations were also noted in a study of young turkeys fed diets with high Lys content and increased levels of Arg (to 110% Lys) and Met (to 45% Lys) (Ognik et al., 2020 b). No such correlations were reported in a previous study of turkeys fed diets with the recommended (NRC, 1994) inclusion rate of Lys (low) and high Arg content (110% Lys) (Ognik et al., 2020 a).

An *in ovo* study of chickens demonstrated that Arg supplementation induces hepatoprotective effects (Toghyani et al., 2019). In an experiment conducted on young turkeys, diets with high Lys content, a decreased content of Arg (to 90% Lys) and an increased content of Met (to 45% Lys) desirably reduced the activity of the liver enzyme AST in the blood plasma, whereas a higher inclusion rate of Arg (100–110% Lys) increased plasma AST levels (Ognik et al., 2020 b). In the present study, diets with a high content of Lys as well as Arg (110% Lys) also increased AST activity in the blood plasma of turkeys, but the inclusion rate of Met had no effect on AST levels. In our previous study of turkeys, an increase in AST activity was not observed when the dietary inclusion rate of Lys was consistent with NRC (1994) recommendations (low) and the inclusion rate of Arg was high (110% Lys) (Ognik et al., 2020 a). The plasma levels of ALT and AST are important indicators of liver health (Pratt and Kaplan, 2000). An increase in ALT or AST activity caused by exposure to high levels of supplemental Arg can be related to hepatocytes' sensitivity to the Arg-induced increase in growth hormone levels (Cravener et al., 1989). Darras et al. (1992) found that liver cells are capable of responding to the growth hormone by converting T_4 to T_3 . According to Carew et al. (1997), the

growth hormone and the thyroid hormone work synergistically. In birds, T_3 is an active thyroid hormone, and peripheral conversion of T_4 to T_3 must occur for the hormone to have a biological effect. The synergistic interactions between growth hormones and T_4 could explain the observed increase in the plasma T_4 levels of turkeys fed diets with the highest inclusion rate of Arg (110% Lys). A similar correlation was noted in a previous study where the Lys content of turkey diets was consistent with NRC (1994) recommendations (low) and the inclusion rate of Arg was high (110% Lys) (Ognik et al., 2020 a). Both T_3 and T_4 stimulate the growth of birds. However, according to Bowen et al. (1984), hyperthyroidism (higher T_4 concentration) decreases and hypothyroidism increases resistance to heat stress and the survival rate of young birds.

Arg contains a basic guanidino group, and it can form chelate rings with certain elements. However, according to Antonilli et al. (2009), Arg does not chelate magnesium (II). By regulating insulin levels, Arg can influence the amount of Mg in cells which is essential for insulin secretion and activity (Kostov, 2019). In the present study, diets with high Lys content (close to BUT recommendations) and the lowest Arg level (90% Lys) decreased the plasma levels of Mg and insulin, whereas the highest Arg level (110% Lys) increased both Mg and insulin concentrations. An increase in the dietary inclusion rate of Met from 30% to 45% of Lys content decreased plasma Mg levels. No such correlations were observed in a previous study where the Lys content of turkey diets was consistent with NRC (1994) recommendations (low) and the inclusion rate of Arg was decreased (90% Lys). An increase in the inclusion rate of Met from 30% to 45% of Lys content did not affect plasma Mg levels (Ognik et al., 2020 a).

In the current experiment, a high dietary inclusion level of Arg (110% Lys) intensified protein nitration and the oxidation of proteins and lipids, as demonstrated by increased levels of 3-NT, PC and MDA. Protein nitration and lipid oxidation were also enhanced when the inclusion of rate of Arg in turkey diets was set at 100% of Lys content. Similar results were noted in another study of young turkeys (Ognik et al., 2020 b). Jankowski et al. (2020 b) reported that different inclusion levels of Arg (90–110% Lys; NRC, 1994) in turkey diets did not induce the oxidation of lipids, proteins or DNA, but protein nitration was intensified when the Arg inclusion rate was increased to 110% of Lys content. An increased supply of Arg promotes the synthesis of NO which activates defense responses in the host organism (which is highly desirable), but it can also pose a certain risk. Nitric oxide is metabolized into highly reactive intermediate products such as peroxynitrite which can initiate lipid peroxidation and thiol oxidation, and disrupt the mitochondrial electron transport chain (Grisham et al., 1999). Nitric oxide can also react with phenol groups, including tyrosine and tryptophan, in selected proteins, which increases 3-nitrotyrosine levels (Kong et al., 1996). According to

some reports, an increase in the dietary content of Arg in excess of the levels recommended by NRC (1994) can stimulate the antioxidant system of birds (Atakisi et al., 2009). However, in the present experiment, an increase in the Arg inclusion rate to 100% of Lys content in diets with high Lys content (close to BUT 2013 guidelines) compromised the antioxidant defense system by increasing MDA levels in the liver, decreasing GSH concentration in the breast muscles, decreasing SOD activity in the blood plasma and intestinal wall, and increasing CAT activity in the breast muscles. In a different study of turkeys where the Lys content of turkey diets was consistent with NRC (1994) recommendations, a decrease in the Arg inclusion rate to 90% of Lys content compromised the antioxidant status of turkeys (Jankowski et al., 2020 b). Hu et al. (2016) found that the supplementation of chicken diets with Arg at 10–25 g/kg did not affect MDA or SOD levels in the liver, but CAT activity in the liver increased in a linear manner with increasing dietary levels of Arg. Catalase activity usually increases in response to intensified oxidation inside cells (Ognik and Krauze, 2016). In the present study, regardless of the Arg inclusion rate, an increase in Met content to 45% of Lys content delivered beneficial effects by decreasing the concentrations of PC, 8-OHdG and 3-NT, and increasing plasma GSH levels. In our previous study, an increase in the inclusion rate of Met from 30% to 45% in diets with a varied content of Arg (90–110% Lys, NRC, 1994) also improved the antioxidant status of turkeys (Jankowski et al., 2020 b; Ognik et al., 2020 a). The stimulatory effect of Met on the antioxidant system of turkeys has been confirmed by many authors (Jankowski et al., 2017 a, b; Zduńczyk et al., 2017; Jankowski et al., 2018).

Conclusions

In growing turkeys fed diets with high Lys content (close to BUT 2013 recommendations), the inclusion rate of Arg can be set at 90% of Lys content with no negative effects on their antioxidant status, blood biochemical parameters or performance. Diets with high Arg content (110% Lys) are not recommended due to the risk of lipid oxidation, protein nitration, and undesirable changes in the concentrations of hormones regulating carbohydrate metabolism, and T_4 levels. Regardless of dietary Arg levels, an increase in the Met inclusion rate from 30% to 45% of Lys content minimizes the oxidation of lipids, proteins and DNA, and increases the antioxidant defense potential of turkeys.

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