Effects of different levels of arginine and methionine in a high-lysine diet on the immune status, performance, and carcass traits of turkeys

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ABSTRACT We postulated that the use of appropriate levels and proportions of arginine (**Arg**) and methionine (**Met**) in compound feed with high lysine content (**Lys**) would make it possible to fully exploit the growth potential of modern fattening turkey crossbreds, without compromising their immune system. The aim of this study was to determine the effect of different ratios of Arg and Met in diets with high Lys content on the performance and immune status of turkeys. The turkeys were assigned to 6 groups with 8 replicates per group and 18 birds per replicate. Six feeding programs, with 3 dietary Arg levels (90, 100, and 110%) and 2 dietary Met levels (30 and 45%) relative to dietary Lys content, were compared. During each of 4 feeding phases (weeks 0–4, 5–8, 9–12, and 13–16), birds were fed ad libitum isocaloric diets containing high level of Lys, approximately 1.83, 1.67, 1.49, and 1.20%, respectively. The dietary treatments had no effect on daily feed intake or body weight at any stage of the study. The protein content of the breast meat was higher in the treatments with the highest Arg level (110%) compared with the lowest Arg level (90%). Similarly, protein content was higher in the treatments with the higher Met level compared with the lower Met level. Higher plasma levels of tumor necrosis factor, interleukin 6 (**IL-6**), and immunoglobulin Y were found in turkeys fed diets with the lowest Arg content. An increase in Met content resulted in a decrease in plasma content of IL-6. In growing turkeys fed diets high in Lys, an Arg level of 90% relative to Lys can be used without negatively affecting production results and immune system. Regardless of dietary Arg levels, an increase in Met content does not stimulate the immune defense system and shows no effect on growth performance of turkeys in current trial.

Key words: Turkey, amino acid, immunity, performance

2020 Poultry Science 99:4730–4740
https://doi.org/10.1016/j.psj.2020.06.039

INTRODUCTION

A diet with the right amino acid profile is crucial to better exploitation of the genetic potential of contemporary, fast-growing crossbreds of fattening turkeys. Owing to the lack of a urea cycle, birds are unable to synthesize arginine (**Arg**), so it is considered an essential amino acid for poultry (Tamir and Ratner, 1963). Studies on broiler chickens have shown a relationship between Arg and lysine (**Lys**) levels in the diet, and any deficiency or surplus of Arg can have a negative effect on levels of this amino acid in the plasma and muscles, and thus on the growth of birds (Balnave and Brake, 2002; Jankowski et al., 2020). However, this effect is more pronounced when the Arg:Lys ratio in the diet is low rather than high (Balnave and Brake, 2002). Arg is a substrate for biosynthesis of creatine, proline, ornithine polyamines, glutamate, and glutamine (Khajali and Wideman, 2010), which are necessary for the normal growth of birds (Chen et al., 2011). In some experiments on broiler chickens, the use of Arg-deficient diets has been found to negatively affect growth performance and to reduce breast and leg muscle mass (Jiao et al., 2010; Khajali et al., 2011). On the other hand, in many experiments on broiler chickens, as well as on White Pekin ducks, Japanese quails, and more recently turkeys, production results and dressing percentage have been improved by using higher Arg levels in the diet than those recommended by the NRC (1994) (Corzo et al., 2003; Fernandes et al., 2009; Wu et al., 2011; Al-Daraji et al., 2011; Al-Daraji and Salih, 2012; Jankowski

Received January 22, 2020.

Accepted June 17, 2020.

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et al., 2020). According to some authors (Gaya et al., 2006; Xiong et al., 2010; Wu et al., 2011), Arg plays a key role in improving the quality of poultry meat by reducing the proportion of undesirable abdominal fat and increasing that of intramuscular fat, which is considered desirable because it improves flavour, juiciness, and water-holding capacity (Hocquette et al., 2010). Many studies summarized in a review by Jankowski et al. (2014), as well as other studies on turkeys (Jankowski et al., 2017a,b), indicate that poultry production can be improved by using an appropriate level of Met in the diet. Arginine can affect turkey growth due to its participation in creatine synthesis. Glycocyamine (a biological precursor of creatine biosynthesis in birds) is synthesized from Arg and Gly. A methyl group is provided to glycocyamine via Met. An increase in dietary Arg content should be accompanied by an increase in Met content, because higher Arg levels contribute to the transfer of methyl groups provided by Met for creatine synthesis. As a result, appropriate methionine and arginine levels improve performance (Jankowski et al., 2020).

Available data indicate that a physiological level of Arg is necessary for innate immune function because it contributes to the maturation and proliferation of T and B cells, which are responsible for the production of cytokines and specific antibodies (Li et al., 2007). Mieulet et al. (2010) have shown that a decrease in extracellular Arg concentration may adversely affect the specific and non-specific immune response. It has been demonstrated that a suitable level of Arg in the diet can increase the weight of the thymus and bursa of Fabricius, thereby improving immunity in birds (Bronte and Zanovello, 2005; Swaggerty et al., 2011), as this amino acid acts as a substrate in the immune system (Wu et al., 2009). Arg stimulates the functions of various cell types in the immune system, including Natural Killer T-cells (**NKT**) (Rhoads et al., 2004). According to Kwak et al. (1999), Arg deficiency in the diet of chickens decreases the relative weight of immunocompetent organs, including the thymus, spleen, and bursa of Fabricius, and impairs immune system efficiency. There are also reports indicating that Met and Lys levels affect the relative weight of lymphoid organs, which are responsible for the formation and maintenance of the B-cell compartment in birds (Jankowski et al., 2014). According to Wu et al. (2013), Met deficiency in the chicken diet inhibits the development of the bursa of Fabricius, while Sigolo et al. (2019) report that increasing the Lys and Met level recommended by the NRC (1994) increases the relative weight of the bursa of Fabricius and thus improves humoral immunity in birds.

Many studies have shown that Arg, Met, and Lys, by modulating the immune system, can have both beneficial and harmful effects on the immune system and on growth performance in poultry if they are used in the diet in incorrect amounts and proportions (Tan et al., 2014, 2015; Hu et al., 2016; Jankowski et al., 2020). Our previous research has shown that feeding growing turkeys a diet with Lys in accordance with NRC (1994) guidelines, that is, a low level, can increase the yield of breast muscles when Arg content is 110% that of Lys (Jankowski et al., 2020). At the same time, our research has shown that when the Lys level in the diet is low, reducing Arg content to 90% that of Lys can adversely affect turkey immunity and growth in the initial rearing period. In addition, irrespective of the Arg content relative to Lys (NRC, 1994), the use of a high level of Met (45% Lys) in the diet was found to improve turkey production results (Jankowski et al., 2020). Both the NRC guidelines (1994) and guidelines that are currently applied to commercial turkeys' nutrition differ regarding essential amino acids level in the diet. For instance, the demands for Lys, Arg, and Met in the first 3 wk of rearing have been set at 1.60, 1.60, and 0.55 of diet, respectively, according to the NRC (1994). But for the same feeding periods, British United Turkeys (BUT) (2013) recommends 1.76, 1.80, and 0.70% of Lys, Arg, and Met levels, respectively, in the turkey diet. Onward feeding phases also vary in these amino acids recommended level in the diet depending on each nutritional guideline. There are several reasons for different recommendations among guidelines including that NRC (1994) recommendations considering the less intensive feeding strategy for birds than that of BUT (2013). The different approach between these guidelines also appear regarding physiological and practical importance of Arg and Met to Lys ratio in the diet for turkeys. Generally, BUT (2013) recommends a higher Arg and Met to Lys ratio (102–105% Lys vs. 90–100% Lys according to NRC (1994)), and the same is true regarding Met level of the Lys level in the diet being 36 to 41% that of Lys according to BUT (2013) and 30 to 38% of the Lys level according to NRC (1994).

We postulated that the use of appropriate levels and proportions of arginine (**Arg**) and methionine (**Met**) in compound feed with high lysine content (**Lys**) would make it possible to fully exploit the growth potential of modern fattening turkey crossbreds, without compromising their immune system. The aim of this study was to determine the effect of different ratios of arginine (Arg) and methionine (Met) in diets with high-lysine (Lys) content on the performance and immune status of turkeys.

MATERIALS AND METHODS

The study protocol was approved by the Local Ethics Committee (University of Warmia and Mazury, Olsztyn, Poland; Resolution no 82/2017), and the birds were cared under guidelines laid down by the EU Directive 2010/63/EU.

Animals and Housing

The birds (864 one-day-old Hybrid Converter female turkey poults) of the initial BW being 57.5 ± 0.5 g were obtained from a commercial hatchery (Grelavi S.A. in Ketrzyn, NE Poland). From the first day of life, birds were randomly allocated in wood shavings littered pens and were assigned to 6 dietary treatments. There were 8 replicate pens per dietary treatment, with each replicate comprising 18 turkeys. The room conditions including temperature and lighting program were consistent with the recommendations of Hybrid Turkeys (BUT, 2013), and the height of the watering and feeding lines was adapted to the birds' growth stage. All birds had unrestricted access to feed and water, which were provided ad libitum throughout the experimental period.

Experimental Design and Diets

The whole feeding program was divided into 4 feeding phases during which each bird was fed isocaloric diets containing a high level of Lys, approximately 1.83, 1.67, 1.48, and 1.20%, respectively, ad libitum. The experimental diets were prepared under the direct supervision of a representative of the Department of Poultry Science, University of Warmia and Mazury at a local feed mill. The following procedure was applied: basal diets without supplemental Lys, Met, and Arg were prepared for each of the feeding phases (Table 1). Then, the amino acid content of the basal diets was determined analytically, and then appropriate amounts of the amino acids were supplemented to reach designed level. Subsequently, the final content of amino acids in the diets was determined analytically again to ensure accuracy. Starter diets (days 1–28) and grower and finisher diets (days 29–112), with no feed additives, were offered as crumbles and pellets (3 mm pellets at 65°C for 45 s), respectively. This experiment had a completely randomized 3 × 2 factorial design, and 3 levels of Arg (90, 100 and 110%) and 2 levels of Met (30 or 45%), relative to the content of dietary Lys, were considered.

Growth Trial and Sample Collection

The following indices were recorded or calculated: 1) BW on a pen basis, 2) daily feed intake per bird (DFI)—calculated on a pen total feed consumption basis for the entire experimental period and for the number of birds and days in the period, and 3) feed conversion ratio (FCR) was calculated based on BWG and feed consumption (kg of feed/kg of BWG). The calculated BWG, DFI, and FCR were adjusted to the weights of dead birds, which were daily recorded.

At 16 wk of life, the blood samples were collected into heparinized tubes from the wing vein and were thereafter centrifuged for 10 min at 3,000 *g* at 4°C. The collected plasma was stored at −20°C for further determination.

At the end of the experiment, birds were weighted after 8-hour feed deprivation, and 1 bird from each

Table 1. Ingredient composition and nutrient content of basal diets (g/100 g, as-fed basis) fed to turkeys at 1–4, 5–8, 9–12, and 13–16 wk of age.

	Feeding period, weeks			
Item	1–4	5–8	9–12	13–16
Ingredients				
Wheat	43.98	47.12	51.99	61.71
Maize	10.00	10.00	10.00	10.00
Soybean meal, 48% CP	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
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	2,820	2,950	3,100	3,150
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

[1]Provided 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. K_3 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.

replicate representing the group average BW was selected and euthanized after electrical stunning. Birds were then hung on a processing line and were bleed out for 3 min by a unilateral neck cut severing the right carotid artery and jugular vein. The non-edible viscera, including intestines, proventriculus, gall bladder, spleen, oesophagus, and full crop, were manually excised after scalding at 61°C for 60 s and defeathering in a rotary drum picker for 25 s. After removing the head, legs, edible viscera (heart, liver, and gizzard), and fat (perivisceral, perineal, and abdominal), carcasses were air pre-chilled at 12°C for 30 min, and then stored at 4°C for 24 h before being hand-deboned on a cone. The carcass yields, including breast muscles (*Pectoralis major* and *Pectoralis minor* muscles) and leg muscles (thigh and drumstick without skin), heart, liver, as well as gizzard weight, and abdominal fat content were calculated relative to the live BW.

Chemical 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 by the method proposed by Moore and Stein (1954). Liquid-phase hydrolysis of powdered samples was performed in 6M HCl containing 0.5% phenol at 110°C for 24 h under an argon atmosphere. The hydrolysates were lyophilized, dissolved in an appropriate volume of dilution buffer, filtered through a 0.45 µm syringe filter, and then applied to the amino acid analyzer. Sulphur-containing amino acids were analyzed as oxidation products obtained by performic acid oxidation (16 h 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). Tryptophan content was determined according to Polish Standard PN-77/R-64820.

During deboning (24 h postmortem), *Pectoralis major* subsamples were taken for determination of the pH and color of the meat. Meat color was determined by the optical reflection method in the CIELAB system (CIE, 1978), with L* (lightness; lower values indicate a darker color), a* (redness; higher positive values indicate more redness), and b* (yellowness; higher positive values indicate more yellowness) measured with a MiniScan XE Plus color difference meter (Hunter Associates Laboratory, Inc., Reston, VA). The average of 2 readings from a cross-section of each right breast muscle, free of color defects, bruising, and hemorrhaging was recorded. Ultimate pH (24 h postmortem) was measured in duplicate at a depth of 2.5 cm below the surface of the left breast muscle, using a Testo 206-pH2 portable pH/°C measuring instrument and a pH2 piercing probe head for semi-solid substances (Testo GmbH and Co., Lenzkirch, Germany).

The content of caspase-3 and caspase-8 was determined in the blood plasma using ELISA kits (Cell Biolabs, Inc. San Diego). The plasma concentrations of immunoglobulins IgA and IgY, interleukins IL-6 and IL-2, TNFα, and globulins were determined in an ELISA reader using assays from Elabscience Biotechnology Co., Ltd. (Houston, Texas). Plasma ceruloplasmin levels were determined using the Ceruloplasmin ELISA kit (Biomatik, Wilmington, DE). Hemoglobin content (**Hb**) was determined in an Abacus Junior Vet hematology analyzer (Diatron, Budapest, Hungary). The plasma content of total protein (**TP**) was measured using an automatic biochemical analyzer (Plasma Diagnostic Instruments Horiba, Kyoto, Japan).

Statistical Analysis

This experiment was performed in a completely randomized 3 × 2 factorial design, and the data (presented as the mean ± SEM) were subjected to 2-way ANOVA to examine the effect of 3 levels of Arg (90, 100, and 110%) and 2 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

Diet Composition

As shown in Table 1, the basal diets without supplemental Lys, Met, and Arg were prepared for each of the 4 feeding phases. Throughout the experiment, the analyzed crude protein content of the basal diets

Table 2. Analyzed amino acid content of basal diets, g/100 g.

	Feeding period, weeks			
Item	1–4	5–8	9–12	13–16
Crude protein	26.57	24.80	22.10	19.15
Aspartic acid	2.41	2.34	1.77	1.44
Glutamic acid	4.50	5.10	4.38	4.30
Serine	1.17	1.30	0.98	0.91
Glycine	1.02	1.13	0.90	0.83
Histidine	0.65	0.67	0.54	0.52
Arginine	1.56	1.42	1.37	1.11
Threonine	1.06	1.11	0.84	0.73
Alanine	1.19	1.23	1.00	0.88
Proline	1.38	1.90	1.44	1.57
Tyrosine	0.96	1.01	0.75	0.66
Valine	1.17	1.21	1.05	0.90
Methionine	0.42	0.39	0.29	0.26
Cysteine	0.42	0.40	0.36	0.33
Isoleucine	1.05	1.06	0.86	0.77
Leucine	2.04	2.16	1.79	1.57
Phenylalanine	1.16	1.29	1.09	0.90
Lysine	1.37	1.21	1.02	0.81
Methionine + cysteine	0.84	0.79	0.65	0.59

(Table 2) was similar to the values calculated based on ingredient composition (Table 1). The content of Lys, Arg, and Met in the basal diets was lower than that recommended by BUT (2013). After supplementation with synthetic Lys, the total content of this amino acid in the experimental diets was approximately 1.83, 1.67, 1.48, and 1.13% in successive months of the experiment (Table 3). After the addition of supplemental Arg and Met, their concentrations in the experimental diets were also close to the values adopted in the experimental design model; minor differences may have been due to analytical error.

Effect on Growth Performance and Carcass Traits of Turkeys

The dietary treatments had no effect on DFI or BW at any stage of the study (Table 4). At 1 to 4 wk of age, FCR was higher in the treatments with Arg100 than in treatments with the lower and higher Arg level (90 and 110% of Lys content; $P = 0.026$). In the other feeding periods and over the entire experiment, the dietary treatments had no influence on FCR. In week 16, the average mortality rate was 1.43%, ranging from 0.7% in treatment Arg90Met45 to 2.0% in treatments Arg90-Met30 and Arg100Met45.

The dietary treatments had no effect on carcass quality parameters, irrespective of Arg and Met levels (Table 5). The yields of whole carcass, breast muscles (*P. major* and *P. minor*), leg muscles (thigh and drumstick without skin), liver and gizzard relative weight, and abdominal fat content were similar for all dietary treatments. There were also no significant differences in the color or pH of the breast meat (Table 6). However, more yellow color in the breast meat was observed in treatments with the higher Met level (45% of Lys content) than in treatments with the lower Met level (30% of Lys content, $P = 0.011$). The protein content of the breast meat was higher in the treatments with the highest Arg level (110% of Lys content) compared with the lowest Arg level ($P = 0.038$). Similarly, protein content was higher in the treatments with the higher Met level (45% of Lys content) compared with the lower Met level ($P = 0.005$) (Table 6).

Effect on the Immune Status of Turkeys

Higher plasma levels of TNFα ($P = 0.028$), IL-6 ($P = 0.045$), and IgY ($P < 0.001$) were found in turkeys fed diets with the lowest Arg content. An increase in Met content from 30 to 45% Lys resulted in a decrease in plasma content of IL-6 ($P = 0.018$). In the case of IgA, there was an Arg × Met interaction ($P = 0.027$), due to the fact that for the lower Met content (30% Lys), increasing Arg from 90 to 110% Lys resulted in a reduction in IgA content, which was not noted in the case of the higher Met content (45% in relation to Lys; Table 7).

A lower Hb level ($P = 0.002$) was found in the blood of turkeys receiving the diet with the lowest Arg level (90% Lys) than in the turkeys receiving a higher Arg level (100 or 110% Lys). Compared to turkeys receiving the intermediate (100% Lys) level of Arg in the diet, the use of the highest content of this amino acid (110% Lys) resulted in a decrease in the plasma level of globulins ($P = 0.032$). Increasing the Met content from 30 to 45% relative to Lys resulted in a decrease in plasma globulin levels ($P = 0.002$). Compared to the lowest and intermediate Arg content (90 and 100% Lys), the highest Arg content (110% Lys) in the diet caused an increase in plasma ceruloplasmin levels ($P = 0.009$; Table 8).

DISCUSSION

In the few previous experiments conducted in turkeys, increasing the Arg:Lys ratio to over 1:1 has been found to improve growth performance (Oso et al., 2017) or has had no effect on growth results (Veldkamp et al., 2005). An experiment conducted on chickens showed that increasing the Arg and Met content in a diet with a high Lys level improves the growth of chicks due to

Table 3. Analysed lysine, arginine, and methionine content of experimental diets fed to turkeys in successive feeding periods, g/100 g.

Feeding period, weeks	AA	Treatment[1]					
		$Arg_{90}Met_{30}$	$Arg_{90}Met_{45}$	$Arg_{100}Met_{30}$	$Arg_{100}Met_{45}$	$Arg_{110}Met_{30}$	$Arg_{110}Met_{45}$
1–4	Lys	1.81	1.82	1.85	1.78	1.89	1.85
	Arg.	1.59	1.64	1.86	1.78	2.04	2.08
	Met	0.53	0.78	0.56	0.76	0.57	0.78
5–8	Lys	1.67	1.70	1.64	1.66	1.65	1.68
	Arg.	1.50	1.48	1.64	1.61	1.77	1.79
	Met	0.50	0.72	0.52	0.71	0.51	0.70
9–12	Lys	1.45	1.50	1.47	1.51	1.48	1.46
	Arg	1.35	1.31	1.50	1.50	1.66	1.65
	Met	0.43	0.67	0.46	0.66	0.46	0.63
13–16	Lys	1.14	1.17	1.16	1.12	1.12	1.09
	Arg	1.03	1.02	1.14	1.11	1.24	1.22
	Met	0.33	0.49	0.34	0.48	0.32	0.49

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Table 4. Daily feed intake (DFI), body weights (BW), and feed conversion ratio (FCR) in turkeys fed diets with different Arg and Met content (weeks 1–16 of age, n = 8).

Item	DFI, weeks (g/bird)					BW, week (kg)				FCR, weeks (kg/kg)					Mortality (%)
	1–4	5–8	9–12	13–16	1–16	4	8	12	16	1–4	5–8	9–12	13–16	1–16	
Treatment[1]															
Arg$_{90}$Met$_{30}$	65.4	195.8	360.1	417.4	239.4	1.25	4.34	8.21	11.43	1.53	1.87	2.47	3.55	2.44	2.0
Arg$_{90}$Met$_{45}$	67.9	200.6	352.4	407.1	239.8	1.29	4.40	8.22	11.45	1.54	1.91	2.46	3.45	2.44	0.7
Arg$_{100}$Met$_{30}$	68.3	195.9	353.8	398.9	237.4	1.28	4.34	8.24	11.38	1.56	1.88	2.42	3.58	2.44	1.3
Arg$_{100}$Met$_{45}$	67.1	200.7	357.7	408.6	239.4	1.26	4.38	8.23	11.43	1.56	1.89	2.49	3.46	2.44	2.0
Arg$_{110}$Met$_{30}$	67.3	196.8	355.3	411.2	240.9	1.29	4.36	8.25	11.47	1.53	1.89	2.46	3.45	2.44	1.3
Arg$_{110}$Met$_{45}$	67.5	201.5	349.4	413.4	238.3	1.28	4.36	8.21	11.49	1.54	1.93	2.44	3.40	2.42	1.3
SEM	0.378	1.682	1.981	2.399	2.083	0.005	0.027	0.036	0.031	0.005	0.008	0.011	0.031	0.011	NA
Arg level, %															
90	66.6	198.2	356.2	412.3	239.6	1.27	4.37	8.21	11.44	1.54[b]	1.89	2.46	3.50	2.44	1.3
100	67.7	198.1	355.6	403.4	238.3	1.27	4.36	8.23	11.40	1.56[a]	1.88	2.45	3.53	2.44	1.7
110	67.4	199.2	352.3	412.3	239.6	1.29	4.36	8.23	11.48	1.54[b]	1.91	2.45	3.42	2.43	1.3
Met level, %															
30	67.0	196.2	356.4	409.2	239.2	1.28	4.34	8.23	11.43	1.54	1.88	2.45	3.53	2.44	1.6
45	67.5	200.9	353.0	409.8	239.1	1.28	4.38	8.21	11.46	1.55	1.91	2.46	3.44	2.43	1.3
P-value															
Arg	0.494	0.969	0.690	0.258	0.967	0.292	0.993	0.974	0.610	0.026	0.478	0.865	0.423	0.947	NA
Met	0.515	0.180	0.425	0.907	0.982	0.669	0.520	0.890	0.625	0.574	0.133	0.477	0.178	0.869	NA
Arg x Met	0.121	0.999	0.466	0.236	0.909	0.053	0.935	0.954	0.988	0.834	0.637	0.163	0.901	0.876	NA

[a-b]Values in the same column with no common superscript denote a significant difference ($P \leq 0.05$).

Abbreviation: NA, not analyzed.

[1]Treatment: Arg$_{90}$Met$_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg$_{90}$Met$_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg$_{100}$Met$_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg$_{100}$Met$_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg$_{110}$Met$_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg$_{110}$Met$_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Table 5. The effect of diets with different Arg and Met content on the carcass traits of turkeys at 16 wk of age (g/100 g body weight).

Item	Live BW (kg)	Dressing yield	Breast muscles	*Pectoralis major*	*Pectoralis minor*	Thigh	Drumstick	Abdominal fat	Heart	Liver	Gizzard
Treatment[1]											
$Arg_{90}Met_{30}$	11.47	82.11	22.38	17.64	4.74	10.40	8.09	1.87	0.29	1.34	0.56
$Arg_{90}Met_{45}$	11.65	82.18	22.49	17.75	4.74	10.68	8.27	1.86	0.29	1.37	0.50
$Arg_{100}Met_{30}$	11.44	82.30	22.47	17.76	4.71	10.67	8.04	1.71	0.33	1.28	0.53
$Arg_{100}Met_{45}$	11.56	82.61	22.22	17.46	4.75	11.03	8.35	1.75	0.30	1.32	0.56
$Arg_{110}Met_{30}$	11.63	82.11	22.13	17.35	4.77	10.77	8.15	1.68	0.31	1.37	0.51
$Arg_{110}Met_{45}$	11.65	82.33	22.30	17.47	4.83	10.99	8.20	1.69	0.31	1.34	0.52
SEM	0.030	0.079	0.103	0.099	0.053	0.098	0.078	0.040	0.006	0.033	0.012
Arg level, %											
90	11.56	82.15	22.44	17.70	4.74	10.54	8.18	1.87	0.29	1.36	0.53
100	11.50	82.46	22.35	17.61	4.74	10.85	8.20	1.73	0.32	1.30	0.54
110	11.64	82.22	22.21	17.41	4.80	10.88	8.17	1.69	0.31	1.35	0.51
Met level, %											
30	11.51	82.17	22.33	17.58	4.74	10.61	8.09	1.75	0.31	1.33	0.53
45	11.62	82.37	22.34	17.56	4.78	10.90	8.27	1.77	0.30	1.34	0.52
P-value											
Arg	0.141	0.260	0.697	0.507	0.858	0.310	0.992	0.178	0.210	0.750	0.566
Met	0.074	0.209	0.959	0.907	0.755	0.154	0.270	0.832	0.416	0.889	0.729
Arg x Met	0.532	0.823	0.690	0.647	0.971	0.958	0.815	0.962	0.519	0.913	0.249

[a-b]Values in the same column with no common superscript denote a significant difference ($P \leq 0.05$).

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

the beneficial interactions of these amino acids (Chamruspollert et al., 2002). In our other experiment on turkeys, in which the Lys content in the diet was in line with the recommendations of NRC (1994), weight gain increased if the Met content in the diet (relative to the Lys level) was increased from 30 to 45% and the Arg content from 90 to 100%. The changes in the amino acid composition of the diet did not affect FCR (Jankowski et al., 2020). In the present study, the differences in Arg and Met levels in the diet relative to the high Lys content (similar to BUT recommendations, 2013) did not affect turkey production results. The survival rate of the turkeys was very high in all groups, exceeding 98%.

Table 6. The effect of diets with different Arg and Met content on the physicochemical properties of breast meat in turkeys at 16 wk of age.

Item	Crude protein, %	Color[2] L*	a*	b*	pH_{24}
Treatment[1]					
$Arg_{90}Met_{30}$	24.74	55.97	5.19	12.17	5.73
$Arg_{90}Met_{45}$	25.17	56.07	5.11	12.71	5.74
$Arg_{100}Met_{30}$	25.12	55.77	5.05	11.65	5.72
$Arg_{100}Met_{45}$	25.35	56.15	5.06	12.77	5.70
$Arg_{110}Met_{30}$	25.13	54.91	5.15	11.99	5.65
$Arg_{110}Met_{45}$	25.49	55.39	5.13	12.15	5.76
SEM	0.064	0.302	0.075	0.058	0.025
Arg level, %					
90	24.96^{b}	56.02	5.15	12.44	5.74
100	$25.23^{a,b}$	55.96	5.05	12.21	5.71
110	25.31^{a}	55.15	5.14	12.07	5.71
Met level, %					
30	25.00^{b}	55.55	5.13	11.94^{b}	5.70
45	25.34^{a}	55.87	5.10	12.54^{a}	5.73
P-value					
Arg	0.038	0.457	0.866	0.411	0.867
Met	0.005	0.611	0.882	0.011	0.525
Arg x Met	0.780	0.968	0.971	0.234	0.589

[a-b]Values in the same column with no common superscript denote a significant difference ($P < 0.05$).

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

[2]L* - lightness, lower values indicate a darker color, a* - redness, higher positive values indicate more red color, b* - yellowness, higher positive values indicate more yellow color.

The results of many experiments indicate that feeding chickens a diet with less Arg than the recommended level (NRC, 1994) results in a significant reduction in breast and leg muscle weight (Jiao et al., 2010; Khajali and Wideman, 2010; Chen et al., 2011). On the other hand, the use of higher Arg levels than recommended by NRC (1994) improved carcass yield and increased breast and leg muscle weight (Al-Daraji et al., 2011; Al-Daraji and Salih, 2012). Our other research in turkeys found that using a diet with higher Arg content than recommended by the NRC (1994), that is, up to 110% relative to Lys, increased the weight of turkey breast meat (Jankowski et al., 2020).

In the present study, the experimental factors did not affect the carcass traits. On the other hand, more yellow color was noted in turkey breast meat from groups with higher Met levels (45% Lys). The increase in yellow color may have been due to increased fat deposition between the muscles, which is desirable for consumers because it improves taste, juiciness, and water-holding capacity (Hocquette et al., 2010).

Many cells of the body use Arg as a substrate for synthesis of nitric oxide (**NO**), which is involved in the immune response and other biochemical processes. The availability of intracellular Arg therefore limits NO synthesis, which is catalyzed by the enzymes arginase and

Table 7. Blood immunological parameters of turkeys at 16 wk of age.

Item	TNFα ng/L	IgA μg/L	IgY mg/L	IL-6 ng/L	IL-2 ng/L
Treatment[1]					
$Arg_{90}Met_{30}$	5.014	10.002[a]	3.358	24.37	37.44
$Arg_{90}Met_{45}$	4.355	8.804[a,b]	3.411	20.78	33.40
$Arg_{100}Met_{30}$	2.742	9.280[a,b]	1.776	21.11	31.81
$Arg_{100}Met_{45}$	2.798	8.903[a,b]	2.300	12.13	28.93
$Arg_{110}Met_{30}$	2.852	7.093[b]	2.015	18.35	27.69
$Arg_{110}Met_{45}$	3.044	9.688[a]	1.517	15.17	28.62
SEM	0.249	0.307	0.153	1.172	1.307
Arg level, %					
90	4.684[a]	9.403	3.385[a]	22.58[a]	35.42
100	2.770[b]	9.092	2.038[b]	16.62[b]	30.37
110	2.948[b]	8.391	1.766[b]	16.76[b]	28.15
Met level, %					
30	3.536	8.792	2.383	21.28[a]	32.31
45	3.399	9.132	2.409	16.03[b]	30.31
P-value					
Arg	0.002	0.355	0.001	0.045	0.071
Met	0.763	0.561	0.909	0.018	0.439
Arg x Met	0.711	0.027	0.202	0.474	0.711

[a,b]Values in the same column with no common superscript denote a significant difference ($P \leq 0.05$).

Abbreviations: IgY, immunoglobulin Y; IL-6, interleukin 6; IL-2, interleukin 2; TNFα, tumour necrosis factor α; IgA, immunoglobulin A.

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

NO synthase (**iNOS**) (Mori and Gotoh, 2000). Both enzymes compete for Arg and thus play an important role in regulating NO synthesis. Our previous research has established that the use of Lys in the diet of turkeys in the amount recommended by the NRC (1994) together with a low level of Arg (90% Lys) increases IL-6 levels and decreases the total amount of globulins in the blood plasma (Jankowski et al., 2020). Similarly, in the present study, there was also a numerical increase in the plasma, as well as other cytokines (IL-2 and TNF-α), in turkeys receiving a diet with high content of Lys (similar to the level recommended by BUT, 2013) and low content of Arg (90% Lys). However, in the context of increased cytokine levels, there was no increase in the level of

Table 8. Blood hematological, biochemical, and immunological parameters of turkeys at 16 wk of age.

Item	Hb g/L	TP g/L	Globulin μg/L	Ceruloplasmin μg/L	Caspase-3 μg/L	Caspase-8 μg/L
Treatment[1]						
$Arg_{90}Met_{30}$	19.69	22.58	994.7	9.60	0.081	7.279
$Arg_{90}Met_{45}$	19.53	20.68	815.0	9.47	0.107	7.288
$Arg_{100}Met_{30}$	21.98	27.44	1,060.4	9.93	0.080	7.518
$Arg_{100}Met_{45}$	22.74	25.77	914.4	8.54	0.093	7.612
$Arg_{110}Met_{30}$	22.63	27.06	898.1	12.42	0.090	7.229
$Arg_{110}Met_{45}$	22.87	22.98	743.7	11.21	0.096	6.045
SEM	0.397	1.025	28.04	0.384	0.005	0.227
Arg level, %						
90	19.61[b]	21.63	904.9[a,b]	9.53[b]	0.094	7.284
100	22.36[a]	26.61	987.4[a]	9.23[b]	0.086	7.565
110	22.75[a]	25.02	820.9[b]	11.82[a]	0.093	6.637
Met level, %						
30	21.43	25.69	984.4[a]	10.65	0.084	7.342
45	21.72	23.14	824.4[b]	9.74	0.099	6.982
P-value						
Arg	0.002	0.135	0.032	0.009	0.819	0.242
Met	0.696	0.215	0.002	0.207	0.162	0.432
Arg x Met	0.871	0.867	0.959	0.738	0.747	0.444

[a-b]Values in the same column with no common superscript denote a significant difference ($P \leq 0.05$).

Abbreviations: Hb, hemoglobin; TP, total protein.

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

ceruloplasmin, an acute phase protein whose level generally increases during inflammatory reactions (Hellman and Gitlin, 2002). Furthermore, an increase in plasma ceruloplasmin levels in turkeys was observed when a higher level of Arg (110% Lys) was used in the diet. The results of the present and previous studies on turkeys (Jankowski et al., 2020) suggest that the use of lower Arg content than 1:1 in relation to Lys induces mechanisms regulating Arg-dependent biochemical reactions in the cell. A high Lys concentration in the cell has been shown to inhibit arginase activity and Arg uptake by all cell types, thereby suppressing NO and iNOS production (Wu and Morris, 1998; Meininger and Wu, 2002). It is likely that a reduction in the Arg:Lys ratio in the cell results in inhibition of NO synthesis reactions, which trigger overproduction of cytokines responsible for controlling the immune response, which is dependent on the level of NO. Well-known inducers of arginase and iNOS include cytokines IL-1, IL-6, TNF-α, and g-IFN, which are released by T lymphocytes and macrophages (Kwak et al., 2001). The increased plasma level of cytokines observed in our study in turkeys is not indicative of immunosuppression, as postulated in our previous work (Jankowski et al., 2020), but of mobilization of the immune system due to the reduced content of Arg relative to Lys in the cell. This is supported by the results of a study by Subramaniyan et al. (2019), which demonstrated that Arg administered *in ovo* does not intensify but weakens inflammatory reactions, inhibiting the production of inflammatory mediators such as pro-inflammatory cytokines and C-reactive protein in chicken serum. Research in mice has also shown that Arg supplementation in the diet reduces expression of the pro-inflammatory cytokine IL-6 (Coburn et al., 2012). Reducing Arg and Met levels in the diet of poultry has also been shown to reduce the total plasma globulin level, and thus the level of some immunoglobulins as well (Jahanian and Khalifeh-Gholi, 2017). In the present study, there was no reduction in the plasma level of globulins in the turkeys receiving Arg at a level of 90% relative to Lys. Moreover, plasma levels of immunoglobulin Y were elevated in the turkeys fed the diet with the lowest Arg level (90% Lys). Among the 3 classes of immunoglobulins (IgM, IgA, and IgY) found in birds, IgY accounts for over 75% of all immunoglobulins, which is why they are also called avian IgG. Many authors have found increased production of antibodies, including IgG and IgM, in chickens receiving higher Arg levels in their diet than recommended by the NRC (1994) (Perez-Carbajal et al., 2010; Emadi et al., 2011; Al-Daraji and Salih, 2012). Our previous research on turkeys found that the use of varied Arg levels from 90 to 110% Lys (NRC, 1994) had no effect on the levels of IgA and IgY, but the lowest Arg content (90% Lys) reduced the total globulin content (Jankowski et al., 2020).

The present study found that the use of a diet containing a high level of Lys and at the same time a high level of Met (45% of the Lys level) resulted in a decrease in globulins and IL-6. No such correlation was noted in the earlier study in turkeys when low Lys levels consistent with NRC (1994) recommendations and high Met levels (45% Lys) were used (Jankowski et al., 2020). Both Met and Lys are involved in antibody synthesis, so their correct levels and proportions in the diet are essential for immune function (Bouyeh, 2012; Jankowski et al., 2014). Many authors have reported increased antibody levels under the influence of higher levels of Met and Lys than recommended by NRC (1994), but not in healthy birds, but usually in vaccinated or infected birds (Mirzaaghatabar et al., 2011; Bouyeh, 2012; Faluyi et al., 2015).

According to Al-Daraji and Salih (2012), increasing the Arg level in the diet of chickens has a beneficial effect on the red blood cell system by increasing the erythrocyte count and hemoglobin and hematocrit levels. The results of our research confirm those findings, as the highest Hb was recorded in the blood of turkeys receiving the highest Arg level (110% Lys). Emadi et al. (2011) have demonstrated that total protein in the plasma of chickens increases in proportion to the increased content of Arg in their diet. In the present study on turkeys, the differences in the Arg level in the diet did not affect total protein in the blood plasma, but when the highest level of this amino acid (110% Lys) was used, protein content increased in the turkey meat. The increased protein content in the turkey muscles was probably due to an increase in the level of creatine, an endogenous Arg metabolite involved in protein metabolism (Khajali and Wideman, 2010; Chen et al., 2011).

CONCLUSIONS

In growing turkeys fed diets high in Lys, an Arg level of 90% relative to Lys can be used without negatively affecting production results and immune system. However, the increased mobilization of the immune system observed in birds from this treatment indicates the need for further research to clarify whether this Arg:Lys ratio will be sufficient in the case of a disease state in turkeys, when there is a need for rapid mobilization of an efficiently functioning immune system. Regardless of dietary Arg levels, an increase in Met content does not stimulate the immune defense system and shows no effect on growth performance of turkeys in current trial.

ACKNOWLEDGMENTS

This work was supported by the National Science Centre, grant no. 2017/27/B/NZ9/01007.

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

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