The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys

Jan Jankowski,[*] Dariusz Mikulski,[*] Marzena Mikulska,[*] Katarzyna Ognik,[†,1] Zuzanna Całyniuk,[†] Emilia Mróz,[*] and Zenon Zduńczyk[‡]

*Department of Poultry Science, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland; †Department of Biochemistry and Toxicology, University of Life Sciences, 20-950 Lublin, Poland; and ‡Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, 10-748 Olsztyn, Poland

ABSTRACT The research hypothesis postulated that the optimal dietary inclusion levels and ratios of lysine (**Lys**), arginine (**Arg**), and methionine (**Met**) can increase the growth potential of hybrid turkeys and limit metabolic disorders that weaken immune function. The experiment was carried out in a full rearing cycle, from 1 to 16 wk of age, in a two-factorial randomized design with 3 levels of Arg and 2 levels of Met (90, 100 and 110% of Arg, and 30 or 45% of Met, relative to the content of dietary Lys), with 6 groups of 8 replicates per group and 18 turkeys per replicate. In the first and second month of rearing, a significant dietary Arg-by-Met interaction was noted for daily feed intake and body weight gain, and a more beneficial effect was exerted by higher Met content and medium Arg content. Throughout the experiment, the higher dietary Met level increased the final body weight (BW) of turkeys ($P = 0.001$). Different dietary Arg levels had no influence on the growth performance of turkeys, but the lowest level decreased dressing yield ($P = 0.001$), and the highest level increased the percentage of breast muscles in the final BW of turkeys ($P = 0.003$). The lowest Arg level (90% of Lys content) undesirably increased the concentration of the proinflammatory cytokine IL-6 ($P = 0.028$) and decreased globulin concentration ($P = 0.001$) in the blood plasma of turkeys. The higher dietary Met level (45% of Lys content) increased plasma albumin concentration ($P = 0.016$). It can be concluded that higher dietary levels of Met (45 vs. 30% of Lys content) and Arg (100 and 110 vs. 90% of Lys content) have a more beneficial effect on the growth performance and immune status of turkeys.

Key words: turkey, amino acid, blood, immunity

INTRODUCTION

The body weights (**BWs**) of modern commercial hybrid turkeys are twice higher than those noted 40 yr ago at the same slaughter age (15–16 wk in female birds, 19–22 wk in male birds) (Ferket, 2004). However, the selection for growth performance in the previous 40–50 generations of turkeys has compromised the disease resistance of birds (Crespo and Shivaprasad, 2003) and increased their susceptibility to heat stress (Lara and Rostagno 2013). On typical poultry farms with high stocking density, turkeys are exposed to various pathogens that are transmitted via air, feed, and water. Production losses can be minimized by enhancing the systemic immunity of birds (accelerating immune system development) as well as their local (intestinal mucosal) immunity (Kogut, 2009). The significance of the aforementioned strategy is exacerbated by the need to reduce the use of antibiotics in poultry farms.

Amino acids are dietary components with immune-enhancing functions (Kidd and Kerr 1998; Jankowski et al., 2017a, b, 2018). According to many authors, selected amino acids (methionine [Met], arginine [Arg], lysine [Lys]) are used for nutritional purposes, but they also regulate various metabolic processes (Mirzaaghatabar et al., 2011; Wu et al., 2012; Jankowski et al., 2016). Amino acids that participate in the regulation of key metabolic pathways are collectively

referred to as functional amino acids (Wu, 2013). This group of amino acids includes Met and Arg.

The results of studies performed on chickens, which have not been fully confirmed in turkeys, indicate that higher dietary ratios of Arg and Met vs. Lys could improve the antioxidant status and immune functions of birds (Lee et al., 2002; Corzo et al., 2003; Oso et al., 2017). According to many researchers, Arg affects lymphocyte and macrophage functions (Kwak et al., 1999, 2001), thus exerting immunomodulatory and anti-inflammatory effects (Wu and Meininger, 2000; Appleton, 2002; Stechmiller et al., 2005; Ren et al., 2014). Met also stimulates immune function in turkeys, which is manifested mostly by changes in T-cell subpopulations (Jankowski et al., 2014; Kubińska et al., 2015, 2016; Zduńczyk et al., 2017).

Lys, Met, and Arg are amino acids that limit the biological value of protein in cereal-soybean meal-based diets for turkeys (NRC, 1994), but the determination of their optimal dietary inclusion rates and ratios stirs much controversy. According to NRC recommendations (1994), the diets fed to turkeys in the first 4 wk of the rearing period should contain approximately 1.60% Lys, 1.60% Arg, and 0.55% Met. The Lys, Arg, and Met requirements of turkeys in the same period are estimated at 1.76, 1.80, and 0.70% according to British United Turkeys (BUT) guidelines (2013), and similar differences can be found in the recommendations for successive stages of rearing. The aforementioned differences can be regarded as different dietary strategies, where lower supplementation levels are recommended by NRC (1994) and higher supplementation levels are recommended by BUT (2013).

Another difference, important from both physiological and practical perspectives, is the recommended dietary ratios of Arg and Met vs. Lys. According to NRC (1994), the dietary inclusion rate of Arg can reach up to 90–100% of Lys content, whereas a higher inclusion rate of Arg (102–105% of Lys content) is recommended by BUT (2013). The Met inclusion level recommended by NRC (1994) is 30–38% of Lys content, and it is higher at 36–41% of Lys content according to BUT guidelines (2013). The aforementioned differences in nutritional guidelines point to insufficient knowledge about dietary supplementation with amino acids, limiting the biological value of protein in turkeys (Lys, Met, and Arg), including their optimal ratios and effects on the metabolism and growth rate of birds.

In view of the aforementioned information, the aim of this study was to determine the effect of different ratios of Arg, Met, and Lys in diets with Lys content consistent with NRC recommendations (1994) on the performance, carcass traits, and immune status of turkeys.

MATERIALS AND METHODS

Animals and Housing

A total of 864 one-day-old Hybrid Converter female turkey poults obtained from a commercial hatchery (Grelavi company in Ketrzyn, NE Poland) were placed in pens on litter (wood shavings) and were randomly allocated to 6 dietary treatments, with 8 replicate pens (4 m^2 each; 2.0 m $\times$ 2.0 m) per treatment and 18 birds per pen. The stocking density at the initial stage of rearing was 4.5 birds/m^2. The initial BW of one-day-old poults was 55.7 $\pm$ 0.1 g. The temperature and lighting programs were consistent with the recommendations for Hybrid Turkeys (2013). The protocol for this 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. Throughout the experiment, all birds had unlimited access to feed and water. The height of the watering and feeding lines was adapted to the birds' growth stage.

Experimental Design and Diets

During each of 4 feeding phases (4 wk each), birds were fed *ad libitum* isocaloric diets containing 1.60, 1.50, 1.30, and 1.00% of Lys, respectively, as per nutrient requirements of turkeys (NRC, 1994). The experiment had a completely randomized 3 $\times$ 2 factorial design with 3 levels of Arg (90, 100, and 110%) and 2 levels of Met (30 or 45%), relative to the content of dietary Lys. 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 4 feeding phases (4 wk each) (Table 1). The amino acid content of basal diets was determined, and then they were mixed with adequate amounts of the aforementioned amino acids. The total content of amino acids in diets was determined analytically. Starter diets (days 1–28) were offered as crumbles, while grower and finisher diets (days 29 to 112) were prepared as 3-mm pellets at 65°C for 45 s. The experimental diet did not contain any feed additives.

Growth Trial and Sample Collection

The BW of birds were recorded and calculated on a pen basis. Daily feed intake (DFI) per bird was calculated on a pen total feed consumption basis for the entire experimental period and for the number of birds and days in the period. Feed conversion ratio (FCR; kg of feed/kg of body weight gain [BWG]) for the experimental period was calculated on a pen basis based on BWG and feed consumption. Mortality rates, including their causes, were recorded daily, and the weights of dead birds were used to adjust average BWG, DFI, and FCR.

Blood samples were collected at 16 wk of age from the wing vein intravitally. 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 obtained plasma was stored at −20°C until analysis.

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.

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
Ingredients				
Wheat	46.37	48.67	53.74	65.63
Maize	10.00	10.00	10.00	10.00
Soybean meal	25.05	23.27	18.73	7.91
Rapeseed meal	3.00	5.00	7.18	7.00
Potato protein	5.52	3.01	-	-
Soybean oil	0.20	2.32	3.53	3.22
Maize gluten meal	5.50	3.50	3.50	3.50
Sodium bicarbonate	0.20	0.20	0.20	0.20
Sodium chloride	0.15	0.16	0.16	0.14
Limestone	2.20	1.86	1.64	1.38
Monocalcium phosphate	1.46	1.29	0.90	0.50
L-Threonine	-	0.07	0.07	0.17
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	2,825	2,900	3,000	3,100
Crude protein	26.5	23.50	20.50	17.00
Arginine	1.44	1.35	1.17	0.89
Lysine	1.28	1.12	0.89	0.64
Methionine	0.45	0.39	0.34	0.29
Met + Cys	0.92	0.82	0.74	0.65
Threonine	1.02	0.95	0.80	0.75
Tryptophan	0.31	0.28	0.24	0.19
Calcium	1.25	1.10	0.95	0.75
Available phosphorus	0.65	0.55	0.47	0.38

[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.

After 16 wk of feeding, 8 turkeys per group (1 bird representing an average BW per pen) were euthanized at the Department's slaughterhouse 8 h after feed withdrawal. The birds 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 (nonedible viscera: intestines, proventriculus, gall bladder, spleen, esophagus, and full crop). Head, legs, edible viscera (heart, liver, and gizzard), and fat (perivisceral, perineal, and abdominal) were removed to obtain carcasses. After evisceration, whole carcasses were air prechilled at 12°C for 30 min, air chilled, stored at 4°C, and hand-deboned on a cone 24 h postmortem. The yields of whole carcass; breast muscles (including the pectoralis major and pectoralis minor muscles) and leg muscles (including the thigh and drumstick without skin); heart, liver, and gizzard weight; and abdominal fat content were determined relative to 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). The liquid-phase hydrolysis of powdered samples was performed in 6 mol 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, and filtered through a 0.45-μm syringe filter before applying to the amino acid analyzer. Sulfur-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 standard protocol of the manufacturer (INGOS, Czech Republic) (Davidson, 2003). Tryptophan content was determined according to Polish Standard PN-77/R-64820.

At the time of deboning (24 h postmortem), pectoralis major subsamples were used to determine the pH and color of 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 a higher contribution of redness), and b* (yellowness, higher positive values indicate a higher contribution of yellowness) measured using a MiniScan XE Plus color difference meter (Hunter Associates Laboratory, Inc., Reston, VA). The average of 2 readings taken from the cross-section of each right breast muscle free from color defects, bruising, and hemorrhages 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 semisolid substances (Testo GmbH & Co., Lenzkirch, Germany).

The content of caspase-3 (MBS261903) and caspase-8 (MBS094470) was determined in the blood plasma using ELISA kits (Cell Biolabs, Inc., San Diego, CA). The plasma concentrations of immunoglobulins IgA and IgY, interleukins IL-6 and IL-2, tumor necrosis factor α, albumins, and globulins were determined in an ELISA reader using assays from Elabscience Biotechnology Co., Ltd. (Houston, TX). Plasma ceruloplasmin levels were determined using the Ceruloplasmin ELISA kit (Biomatik, Wilmington, DE).

Statistical Analysis

For performance parameters, a single pen or cage (n = 8) was considered as a replicate experimental unit in the statistical analysis. The model assumptions of normality and homogeneity of variance were examined by the Shapiro-Wilk and Levene tests, respectively. The experiment had a completely randomized 3 × 2 factorial design, and two-way ANOVA was performed to assess the effects of diets (with 3 levels of Arg [90, 100, and 110%] and 2 levels of Met [30 or 45%]). When a significant interaction effect was noted (F test),

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

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
Crude protein	27.08	24.63	20.93	17.70
Aspartic acid	2.540	2.122	1.788	1.166
Glutamic acid	5.373	4.606	4.184	3.712
Serine	1.366	1.147	1.013	0.772
Glycine	1.142	0.972	0.869	0.669
Histidine	0.649	0.567	0.518	0.444
Arginine	1.481	1.370	1.193	0.916
Threonine	1.061	0.909	0.768	0.676
Alanine	1.327	1.104	0.987	0.800
Proline	1.815	1.589	1.492	1.460
Tyrosine	0.870	0.819	0.751	0.541
Valine	1.350	1.134	0.984	0.734
Methionine	0.456	0.394	0.336	0.247
Cysteine	0.462	0.409	0.377	0.334
Isoleucine	1.193	0.983	0.868	0.623
Leucine	2.424	1.976	1.733	1.325
Phenylalanine	1.456	1.191	1.035	0.781
Lysine	1.296	1.189	0.962	0.622
Methionine + cysteine	0.918	0.803	0.713	0.581

treatment means were separated using the *post-hoc* Newman-Keuls test. All calculations were performed using the GLM procedures of the STATISTICA software system, version 12.0 (StatSoft Inc., 2014, Tulsa, OK). Data variability was expressed as mean values with a pooled standard error of the mean, and $P < 0.05$ was considered statistically significant.

RESULTS

Diet Composition

Throughout the experiment, the CP content of experimental diets (Table 2) exceeded the values calculated based on diet composition by the amount of supplemental amino acids added to basal diets (Table 1). In basal diets (Table 2), the content of Lys, Agr, and Met was lower than that recommended by the NRC (1994). After supplementation with synthetic Lys, the total content of this amino acid in experimental diets

approximated 1.60, 1.50, 1.30, and 1.00% in consecutive weeks of the experiment (Table 3). 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, and minor differences could be due to analytical error.

Effect on the Growth Performance and Carcass Traits of Turkeys

The applied dietary treatments affected DFI in the first stage of the study, and the observed differences resulted from a significant interaction between Arg and Met levels (Table 4). In the period of 1–4 wk of age, DFI was higher in treatment Arg100Met30 than in treatments Arg90Met30 and Arg110Met30, and it was still higher in treatment Arg110Met45 where the diet contained the highest levels of Arg and Met (Arg × Met interaction, $P = 0.003$). In the period of 5–8 wk of age, DFI increased in the following order: Arg90Met30 < Arg100Met30 and Arg110Met30 < Arg90Met45, Arg100Met45, and Arg110Met45. In the period of 9–12 wk of age, DFI was higher in treatments with a lower Met level (30% of Lys content) than in treatments with the higher Met level (45% of Lys content, $P = 0.035$). In the period of 13–16 wk of age, the dietary treatments had no influence on DFI. Over the entire experiment, DFI was lowest in treatment Arg90Met30, with a numerical difference relative to treatment Arg110Met30 and statistically significant differences ($P = 0.035$) relative to the remaining treatments which did not differ significantly from treatment Arg110Met30 (Arg × Met interaction, $P = 0.008$).

Similar to DFI, differences in the BW of turkeys in weeks 4, 8, and 12 of the experiment resulted from the interaction between Met and Arg levels ($P = 0.019$, $P = 0.024$, and $P = 0.022$, respectively). In week 4, BW was highest in the treatments with a higher Met level regardless of the dietary level of Arg. In weeks 8 and 12, BW was higher in the treatments with higher

Table 3. Lys, Arg, and Met content of experimental diets fed to turkeys in successive feeding periods, g/100 g.

Feeding period, weeks	AA	Treatment[1]					
		$Arg_{90}Met_{30}$	$Arg_{90}Met_{45}$	$Arg_{100}Met_{30}$	$Arg_{90}Met_{45}$	$Arg_{110}Met_{30}$	$Arg_{110}Met_{45}$
1–4	Lys	1.63	1.56	1.58	1.66	1.55	1.64
	Arg	1.46	1.43	1.52	1.56	1.69	1.67
	Met	0.50	0.69	0.51	0.71	0.52	0.74
5–8	Lys	1.48	1.45	1.53	1.56	1.56	1.55
	Arg	1.37	1.39	1.53	1.56	1.71	1.73
	Met	0.42	0.66	0.42	0.70	0.44	0.66
9–12	Lys	1.27	1.29	1.34	1.28	1.36	1.32
	Arg.	1.18	1.20	1.34	1.26	1.45	1.43
	Met	0.38	0.61	0.41	0.59	0.38	0.59
13–16	Lys	1.01	0.99	1.03	1.04	0.96	0.97
	Arg.	0.92	0.91	1.05	1.03	1.12	1.16
	Met	0.33	0.46	0.31	0.42	0.32	0.48

Abbreviations: AA, amino acids; Arg, arginine; Lys, lysine; Met, methionine.

[1]Treatment: 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.

Table 4. Daily feed intake (DFI), body weights (BW), and feed conversion ratio (FCR) in turkeys fed with diets containing 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	Total	4	8	12	16	1–4	5–8	9–12	13–16	Total	
Treatment[1]															
$Arg_{90}Met_{30}$	46.7[c]	134[c]	293	423	214[b]	0.89[c]	2.92[c]	6.64[c]	10.2	1.57	1.88	2.21	3.26	2.43	4.86
$Arg_{90}Met_{45}$	52.3[a,b]	169[a]	315	423	230[a]	1.04[a]	3.67[a]	7.35[a]	10.7	1.50	1.83	2.42	3.44	2.48	2.08
$Arg_{100}Met_{30}$	50.3[b]	145[b]	313	434	227[a]	0.95[b]	3.19[b]	6.96[b]	10.6	1.58	1.85	2.32	3.28	2.47	1.39
$Arg_{100}Met_{45}$	52.0[a,b]	164[a]	317	423	230[a]	1.03[a]	3.71[a]	7.35[a]	10.7	1.50	1.76	2.42	3.46	2.47	2.08
$Arg_{110}Met_{30}$	47.4[c]	145[b]	309	433	222[a,b]	0.91[b,c]	3.12[b]	6.96[b]	10.5	1.57	1.88	2.28	3.38	2.48	2.78
$Arg_{110}Met_{45}$	53.0[a]	167[a]	309	423	229[a]	1.03[a]	3.71[a]	7.34[a]	10.6	1.52	1.77	2.37	3.50	2.47	2.08
SEM	0.43	2.11	2.21	2.21	1.20	0.01	0.05	0.05	0.04	0.01	0.01	0.02	0.02	0.01	NA
Arg level, %															
90	49.5	151	304	423	222	0.96	3.30	6.99	10.5	1.53	1.85	2.32	3.35	2.46	3.47
100	51.2	155	315	428	229	0.99	3.45	7.15	10.6	1.54	1.81	2.37	3.37	2.47	1.73
110	50.2	156	309	428	225	0.97	3.42	7.15	10.5	1.54	1.83	2.33	3.44	2.47	2.43
Met level, %															
30	48.2	141	305[b]	430	221	0.92	3.08	6.85	10.4[b]	1.57[a]	1.87[a]	2.27[b]	3.30[b]	2.46	3.00
45	52.4	167	314[a]	423	230	1.03	3.70	7.34	10.7[a]	1.51[b]	1.79[b]	2.40[a]	3.47[a]	2.47	2.08
P value															
Arg	-	-	0.091	0.519	-	0.127	-	-	0.106	0.668	0.126	0.118	0.090	0.620	NA
Met	-	-	0.035	0.126	-	-	-	-	0.001	0.001	0.001	0.001	0.001	0.342	NA
Arg × Met	0.003	0.001	0.054	0.532	0.008	0.019	0.024	0.022	0.052	0.384	0.523	0.078	0.703	0.083	NA

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

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; NA, not analyzed; SEM, standard error of mean.

[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.

Met level, regardless of Arg levels. The lower Met level led to a decrease in BW in treatments Arg110Met30 and Arg100Met30, and its further decrease in treatment Arg90Met30. In week 16, the average BW of turkeys was higher in treatments with a higher Met level (45% of Lys content) than in those with a lower Met level (30% of Lys content, $P = 0.001$). During this period, P value for the Arg $\times$ Met interaction was close to being significant ($P = 0.052$).

Dietary Arg levels had no effect on FCR, whereas Met levels exerted varied effects in the first and second stage of the study. In the first stage (1–8 wk of age), the higher Met level improved FCR, and the reverse was noted in the second stage (9–16 wk of age). The difference in the average BW of turkeys noted between treatments Met30 and Met45 decreased in weeks 12 and 16, compared with that in week 8. Throughout the experiment, FCR was not affected by different dietary Arg and Met levels, relative to Lys. In week 16, the average mortality and culling rate was 2.54%, ranging from 1.39% in treatment Arg100Met30 to 4.86% in treatment Arg90Met30.

The applied dietary treatments influenced carcass dressing percentage and the percentage of breast muscles in the carcass, whereas they had no effect on the remaining carcass quality parameters (Table 5). Dressing yield was lower in the treatments with the lowest Arg level (90% of Lys content) than in treatments with the medium and highest Arg levels ($P = 0.001$). The proportion of breast muscles was higher in the treatments with the highest Arg level ($P = 0.001$) than that in those with the medium and lowest Arg levels. The breast meat response was exclusively due to increased weight of the pectoralis major, whereas the

pectoralis minor remained unaffected. The protein content, color, and pH of breast meat were similar in all dietary treatments, regardless of Arg and Met levels (Table 6).

Effect on the Immune Status of Turkeys

Different dietary Arg levels, relative to Lys, did not affect the concentrations of tumor necrosis factor α, IgA, IgY, IL-2, or caspase-8 (Table 7). Turkeys fed with diets containing the lowest Arg level were characterized by higher concentration of the proinflammatory cytokine IL-6 ($P = 0.028$). An increase in Met content from 30 to 45% of Lys content led to an increase in plasma albumin concentration ($P = 0.016$), and the lowest Arg level decreased plasma globulin concentration ($P < 0.001$) (Table 8). Blood ceruloplasmin concentration was highest in treatment $Arg_{110}Met_{45}$ because of the interaction between the higher Met level and the highest Arg level ($P = 0.008$). The highest Arg level caused an increase in caspase-3 concentration ($P < 0.001$), in comparison with the medium and lowest levels of this amino acid. A similar trend was noted in caspase-8, whose concentration was numerically higher ($P = 0.059$) in turkeys fed with diets containing the highest Arg level than in those receiving diets with the lowest Agr level.

DISCUSSION

Effect on the Growth Performance and Carcass Traits of Turkeys

A few experiments performed on turkeys whose diets were formulated based on NRC recommendations

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, total	Pectoralis major	Pectoralis minor	Thigh	Drumstick	Abdominal fat	Heart	Liver	Gizzard
Treatment[1]											
$Arg_{90}Met_{30}$	10.32	82.0	23.1	18.4	4.67	10.8	8.11	1.68	0.31	1.72	0.67
$Arg_{90}Met_{45}$	10.80	82.3	22.8	18.1	4.63	10.6	8.01	1.83	0.30	1.75	0.62
$Arg_{100}Met_{30}$	10.53	82.8	22.7	18.2	4.50	11.0	8.15	1.90	0.32	1.74	0.66
$Arg_{100}Met_{45}$	10.54	82.8	23.5	19.0	4.57	10.6	7.96	1.78	0.30	1.59	0.64
$Arg_{110}Met_{30}$	10.25	83.1	24.0	19.3	4.66	10.6	8.03	1.66	0.31	1.53	0.67
$Arg_{110}Met_{45}$	10.76	83.2	24.0	19.4	4.62	10.3	7.96	1.91	0.30	1.63	0.62
SEM	0.063	0.110	0.132	0.122	0.031	0.084	0.068	0.250	0.004	0.030	0.011
Arg level, %											
90	10.56	82.2[b]	22.9[b]	18.26[b]	4.65	10.7	8.06	1.75	0.31	1.74	0.65
100	10.54	82.8[a]	23.1[b]	18.60[b]	4.54	10.8	8.06	1.84	0.31	1.67	0.65
110	10.51	83.1[a]	24.0[a]	19.34[a]	4.64	10.4	7.99	1.78	0.30	1.58	0.65
Met level, %											
30	10.37	82.6	23.3	18.66	4.61	10.8	8.10	1.75	0.31	1.67	0.67
45	10.70	82.8	23.4	18.82	4.60	10.5	7.98	1.84	0.30	1.66	0.63
P value											
Arg	0.932	0.001	0.001	0.003	0.276	0.163	0.914	0.599	0.898	0.097	0.992
Met	0.008	0.512	0.513	0.452	0.936	0.077	0.401	0.221	0.274	0.851	0.115
Arg $\times$ Met	0.159	0.759	0.135	0.133	0.746	0.801	0.933	0.104	0.876	0.197	0.838

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

Abbreviations: Arg, arginine; BW, body weight; Lys, lysine; Met, methionine; SEM, standard error of mean.

[1]Treatment: 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.

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] | | | pH$_{24}$ |
		L*	a*	b*	
Treatment[1]					
Arg$_{90}$Met$_{30}$	24.9	58.7	5.49	11.6	5.97
Arg$_{90}$Met$_{45}$	25.1	58.7	5.39	11.9	5.66
Arg$_{100}$Met$_{30}$	24.9	61.1	5.20	12.1	5.72
Arg$_{100}$Met$_{45}$	25.0	60.0	5.04	11.6	5.71
Arg$_{110}$Met$_{30}$	24.9	60.0	5.15	11.4	5.71
Arg$_{110}$Met$_{45}$	25.2	58.9	5.10	11.6	5.73
SEM	0.054	0.370	0.092	0.137	0.044
Arg level, %					
90	250	58.7	5.44	11.8	5.81
100	25.0	60.5	5.12	11.8	5.71
110	25.1	59.4	5.13	11.5	5.72
Met level, %					
30	24.9	59.9	5.28	11.7	5.80
45	25.1	59.2	5.18	11.7	5.70
P value					
Arg	0.759	0.137	0.300	0.572	0.557
Met	0.204	0.335	0.583	0.982	0.245
Arg × Met	0.815	0.801	0.973	0.467	0.219

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; SEM, standard error of mean.

[1]Treatment: 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.

[2]L*: lightness, lower values indicate a darker color; a*: redness, higher positive values indicate a higher contribution of redness; b*: yellowness, higher positive values indicate a higher contribution of yellowness.

Table 7. Blood immunological parameters of turkeys at 16 wk of age fed with diets containing different Arg and Met content.

Item	TNFα pg/mL	IgA ng/mL	IgY μg/mL	IL-6 pg/mL	IL-2 pg/mL
Treatment[1]					
Arg$_{90}$Met$_{30}$	40.26	0.749	1.470	1.807	6.72
Arg$_{90}$Met$_{45}$	40.59	0.774	1.527	1.718	6.32
Arg$_{100}$Met$_{30}$	48.76	0.810	1.497	1.207	5.40
Arg$_{100}$Met$_{45}$	49.03	0.742	1.623	1.415	6.62
Arg$_{110}$Met$_{30}$	45.47	0.830	1.556	1.295	5.76
Arg$_{110}$Met$_{45}$	41.35	0.776	1.012	1.467	6.11
SEM	1.855	0.023	0.079	0.074	0.197
Arg level, %					
90	40.42	0.761	1.499	1.762[a]	6.52
100	48.90	0.776	1.560	1.311[b]	6.01
110	43.41	0.803	1.284	1.381[b]	5.94
Met level, %					
30	44.83	0.796	1.508	1.436	5.96
45	43.65	0.764	1.3878	1.533	6.35
P value					
Arg	0.187	0.775	0.319	0.028	0.422
Met	0.756	0.507	0.441	0.500	0.319
Arg × Met	0.858	0.700	0.163	0.652	0.252

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

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; SEM, standard error of mean; TNFα, tumor necrosis factor α.

[1]Treatment: 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.

(1994) reported that increasing dietary inclusion levels of Met exerted varied effects on the growth rate of turkeys, FCR, and carcass quality (Jankowski et al., 2016; Murawska et al., 2018). An increase in the dietary Arg:Lys ratio above 1:1 also produced divergent results: it improved turkey growth (Oso et al., 2017), but only in diets that were deficient in Arg and contained low levels of Lys (Waldroup et al., 1998; Wu et al., 2012) or had no influence on the growth performance of birds (Veldkamp et al., 2005). Arg is an amino acid that produces creatine and enhances the release of insulin-like growth factor 1 in muscles (Newsholme et al., 2005). Chamruspollert et al. (2002) demonstrated that in diets rich in Lys, an increase in Arg and Met levels improved the growth rate of chicks because of beneficial interactions between these amino acids. Other authors (Jahanian, 2009; Chen et al., 2011; Al-Daraji and Salih 2012) also found that diets with increased Arg content and Met and Lys levels consistent with NRC recommendations (1994) improved the growth rates of chickens and turkeys. Also other studies of broiler chickens (Chamruspollert et al., 2002; Khalifeh-Gholi and Jahanian, 2012) and turkeys (Jahanian and Khalifeh-Gholi 2017) revealed strong interactions between Arg and Met, which increased the growth rate of birds. In our study, throughout the rearing period, BW increased in the treatments in which Met level was increased from 30 to 45% of Lys content and Arg level

was increased from 90 to 100% of Lys content. The changes in the amino acid composition of diets had no influence on FCR.

Similar to the results reported for broiler chickens by Chamruspollert et al. (2002), in this study, DFI and the growth rate of young turkeys (1–8 wk of age) were affected not only by Arg and Met vs. Lys ratios but also by the Arg:Met ratio. The lower Met level (30% of Lys content) and the highest Arg level (110% of Lys content) decreased the growth performance of turkeys compared with the higher Met level (45% of Lys content) and the optimal Arg level (100% of Lys content). As a substrate for protein biosynthesis, Arg can affect turkey growth. It also stimulates the pituitary gland and spleen to secrete hormones, that is, insulin, glucagon, and growth hormone, thus enhancing protein biosynthesis (Jahanian and Khalifeh-Gholi 2017). Arg is also involved in the synthesis of ornithine, a polyamine precursor. Therefore, Arg derivatives can stimulate DNA synthesis and cell proliferation. Arg can also affect turkey growth because of its participation in creatine synthesis (Calder et al., 2002). According to Jahanian and Khalifeh-Gholi (2017), creatine synthesis is one of the major mechanisms involved in Arg and Met interactions. Glycocyamine (a biological precursor of creatine biosynthesis in birds) is synthesized from Arg and Gly. A methyl group is provided to glycocyamine via Met. Thus, an increase in dietary Arg content relative to Lys content should be accompanied by an increase in Met content relative to Lys content because higher Arg levels

Table 8. Blood immunological parameters of turkeys at 16 wk of age fed with diets differing in Arg and Met content.

Item	Albumin g/L	Globulin ng/mL	Ceruloplasmin ng/mL	Caspase-3 ng/mL	Caspase-8 ng/mL
Treatment[1]					
Arg90Met30	17.41	18.79	0.803[b]	0.032	0.773
Arg90Met45	21.20	19.69	1.122[b]	0.035	0.794
Arg100Met30	18.84	24.52	0.890[b]	0.043	0.856
Arg100Met45	20.14	27.05	0.892[b]	0.039	0.897
Arg110Met30	19.87	25.38	0.914[b]	0.057	0.902
Arg110Met45	22.74	25.52	1.637[a]	0.058	0.975
SEM	0.552	0.812	0.054	0.002	0.026
Arg level, %					
90	19.30	19.24[b]	0.963	0.033[b]	0.784
100	19.49	25.78[a]	0.891	0.041[b]	0.876
110	21.30	25.45[a]	1.276	0.057[a]	0.938
Met level, %					
30	18.71[b]	22.90	0.869	0.044	0.844
45	21.36[a]	24.09	1.217	0.044	0.889
P value					
Arg	0.239	0.001	-	<0.001	0.059
Met	0.016	0.409	-	0.971	0.390
Arg × Met	0.619	0.784	0.001	0.700	0.917

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

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; SEM, standard error of mean.

[1]Treatment: 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.

contribute to the transfer of methyl groups provided by Met for creatine synthesis. As a result, Met deficiency can decrease the growth performance of turkeys.

In our experiment, no differences in mortality rates were observed between dietary treatments. Different results were noted in earlier studies of broiler chickens where an increase in the dietary Arg:Lys ratio above 1:1 improved the health status of birds and reduced mortality (Kidd et al., 2002; Corzo et al., 2003).

In the present experiment, different dietary Agr and Met levels had no effect on the proportions of most muscles (except breast muscles) and internal organs in the carcass or on the color, pH, and protein content of breast muscles. The medium and highest Arg levels had a beneficial influence on dressing yield compared with the lowest Arg level (90% of Lys content). The highest Arg level (110 of Lys content) increased the percentage of breast muscles in the carcass compared with the medium and lowest Arg levels. Increasing dietary Arg levels increased the yield of pectoralis major but not pectoralis minor, which is consistent with the findings of Lehmann et al. (1997) who investigated the effects of dietary threonine in turkeys. In one of the few experiments performed on turkeys, an increase in the dietary Arg:Lys ratio above 1:1 increased the percentage of breast muscles in the carcass (Kidd and Kerr, 1998), which could be related to the fact that Arg enhances glucose uptake by muscle cells, thus contributing to muscle growth and increasing muscle yield (Stevens et al., 2000). Arg is also

a precursor in the synthesis of important metabolic components, including nitric oxide (**NO**) (Kim et al., 2007). NO relaxes vascular smooth muscles, which promotes blood flow and the supply of oxygen, glycogen, amino acids, creatine, and essential nutrients to the muscles, thus increasing muscle weight (Stevens et al., 2000; Uni and Ferket, 2003).

Effect on the Immune Status of Turkeys

Arg plays an important role in both innate and acquired immunity in poultry because it affects cytokine production, lymphocyte proliferation, and the synthesis of specific antibodies (Kwak et al., 2001; Kidd et al., 2001; Jahanian, 2009; Ren et al., 2014). In the present study, the lowest dietary Arg level (90% of Lys content) increased the concentration of the proinflammatory cytokine IL-6 in the blood plasma of turkeys. A decrease in extracellular Arg concentration can weaken the innate immune response by impairing the specific mitogen-activated protein kinases (**MAPKs**) pathway downstream of the Toll-like receptor 4 signaling (Mieulet et al., 2010). In this signal transduction pathway, Arg prevents the dephosphorylation of tumor-promoting locus 2 (a MAPK kinase) via yet unknown mechanisms, leading to the activation of MAPK and the subsequent production of proinflammatory cytokines (Morris, 2010). In our study, the lowest dietary Arg content decreased plasma γ-globulin levels.

According to many researchers, dietary Arg and Met deficiencies decrease plasma γ-globulin levels in poultry (Jahanian 2009; Jahanian and Khalifeh-Gholi 2017). As the γ-globulin fraction of blood plasma contains immunoglobulins (Tizard, 2012), a decrease in the concentrations of these proteins due to dietary Arg deficiency weakens immune function in turkeys. According to Qureshi (2003) and Xu et al. (2018), Arg is a limiting substrate for NO synthesis, and NO is a paracrine mediator of several immune functions of macrophages in birds. Arg, a substrate for the synthesis of endogenous NO, plays an important role in cell apoptosis, next to ornithine and polyamine, because NO inhibits apoptosis by suppressing caspase activity. In general, low (physiological) concentrations of NO inhibit apoptosis, whereas too high concentrations of this gas may induce programmed cell death (Lind, 2004). In the present study, the highest Arg level (110% of Lys content) increased the plasma concentrations of caspase-3; a numerical (nearly significant) increase was also noted in the plasma concentrations of caspase-8. An analysis of cell lines revealed that N Ω-hydroxy-L-arginine, arginase inhibitor, activates caspase-3 and apoptosis but only at a considerable decrease in intracellular polyamine levels (Singh et al., 2000).

In this experiment, the higher level of Met (45 vs. 30% of Lys content) increased plasma albumin concentration. Albumin, which accounts for 55 to 60% of total plasma protein, performs important physiological functions: it maintains oncotic pressure and is responsible for the transport of selected hormones and fatty acids. Albumin

consists of 585 amino acids arranged in helices that are held by 17 disulfide bridges from sulfur-containing amino acids: cystine, cysteine, and Met. Plasma albumin is considered an important indicator of the nutritional status (Fuhrman, 2002). Increased amino acid availability represents an important regulator of protein synthesis via enhanced translational initiation and promotion of translational elongation (Hülshoff et al., 2013). In the context of our findings, it seems that Arg content cannot be reduced to 90% Liz because this may result in a deterioration of turkey immunity, and a slight improvement in immunity can be obtained when we increase the Arg ratio to 110% Liz and Met supplementation represents 45% of Lys content.

CONCLUSIONS

The higher dietary Met level (45 vs. 30% of Lys content) increased the final BW of turkeys and caused beneficial increased plasma albumin concentration. Throughout the experiment, different dietary Arg levels had no influence on the growth performance of turkeys. However, in younger birds (1–8 wk of age), the lowest Arg level (90% of Lys content) decreased BW and dressing yield at the end of rearing and weakened the immune function of turkeys by decreasing globulin concentration and increasing the concentration of the proinflammatory cytokine IL-6 in their blood plasma. The highest Arg level (110% of Lys content) exerted a beneficial effect by increasing the percentage of breast muscles in the final BW of turkeys and the plasma concentrations of caspase-3 and caspase-8.

ACKNOWLEDGMENTS

REFERENCES

Al-Daraji, H. J., and A. M. Salih. 2012. The influence of dietary arginine supplementation on blood traits of broiler chickens. Pak. J. Nutr. 11:258–264.

AOAC 2005. Official Methods of Analysis of the Association of the Official Analytical Chemists, 18th ed. AOAC International, Arlington, VA.

Appleton, J. 2002. Clinical potential of a semi-essential amino acid. Altern. Med. Rev. 7:512–522.

British United Turkeys (BUT): Aviagen Turkeys 2013. Management guidelines for raising commercial turkeys. Accessed October 2013. https://www.aviagenturkeys.com/media/183481/aviagen commer cial guide.pdf.

CIE 1978. Recommendations on uniform color spaces-color difference equations. Psychometric Color Terms. Supplement No. 2 to CIE Publication No. 15 (E-1.3.1), 1971/(TC-1-3). Commission Internationale de l'E A clairage, Paris.

Calder, P. C., C. J. Field, and H. S. Gill. 2002. Nutrition and Immune Function. CABI Publishing in Association with the Nutrition Society, Wallingford, UK.

Chamruspollert, M., G. M. Pesti, and R. I. Bakalli. 2002. Dietary interrelationships among arginine, methionine, and lysine in young broiler chicks. Br. J. Nutr. 88:655–660.

Chen, J., M. Wang, Y. Kong, H. Ma, and S. Zou. 2011. Comparison of the novel compounds creatine and pyruvate on lipid and protein metabolism in broiler chickens. J. Anim. Sci. 5:1082–1089.

Corzo, A., E.T. Moran, Jr, and D. Hoehler. 2003. Arginine need of heavy broiler males: applying the ideal protein concept. Poult. Sci. 82:402–407.

Crespo, R., and H. L. Shivaprasad. 2003. 2003. Developmental, Metabolic, and Other Noninfectious Disorders, Diseases of Poultry, 11th ed. Iowa State Press, IA.

Davidson, I. 2003. Hydrolysis of samples for amino acid analysis. Methods in molecular biology. In: Protein Sequencing Protocols, vol. 211. Humana, Totowa, NJ.

Ferket, P. S. 2004. Tom Weights up Seven Percent. WATT Poultry USA.

Fuhrman, M. P. 2002. The albumin-nutrition connection: separating myth from fact. Nutrition 18:199–200.

Hülshoff, A., T. Schricker, H. Elgendy, R. Hatzakorzian, and R. Lattermann. 2013. Albumin synthesis in surgical patients. Nutrition 29:703–707.

Jahanian, R., and M. Khalifeh-Gholi. 2017. Marginal deficiencies of dietary arginine and methionine could suppress growth performance and immunological responses in broiler chickens. J. Anim. Physiol. Anim. Nutr. 102:e11–e12.

Jahanian, R. 2009. Immunological responses as affected by dietary protein and arginine concentrations in starting broiler chicks. Poult. Sci. 88:1818–1824.

Jankowski, J., K. Ognik, A. Koncicki, M. Kubińska, Z. Zduńczyk, and B. Tykałowski. 2018. The effect of different dietary levels DL-methionine and DL-hydroxy analog on antioxidant status of young turkeys infected of the haemorrhagic enteric adenovirus isolate. BMC Vet. Res. 14:1–8.

Jankowski, J., K. Ognik, M. Kubińska, A. Czech, J. Juśkiewicz, and Z. Zduńczyk. 2017b. The effect of different dietary levels and sources of methionine on the metabolic parameters, redox status, immune response and growth performance of turkeys. Poult. Sci. 96:3229–3238.

Jankowski, J., M. Kubińska, and Z. Zduńczyk. 2014. Nutritional and immunomodulatory function of methionine in poultry diets a review. Ann. Anim. Sci. 14:17–31.

Jankowski, J., M. Kubińska, J. Juśkiewicz, A. Czech, and Z. Zduńczyk. 2016. The effect of dietary methionine levels on fattening performance and selected blood and tissue redox parameters. Arch. Anim. Nutr. 70:127–140.

Jankowski, J., M. Kubińska, J. Juśkiewicz, A. Czech, K. Ognik, and Z. Zduńczyk. 2017a. Effect of different dietary methionine levels on the growth performance and tissue redox parameters. Poult. Sci. 96:1235–1243.

Khalifeh-Gholi, M., and R. Jahanian. 2012. Immune functions as affected by dietary arginine by methionine interaction in broiler chicks. World's Poult. Sci. J. 68:1–4.

Kidd, M. T., and B. J. Kerr. 1998. Dietary arginine and lysine ratios in large white toms. 2. lack of interaction between arginine: lysine ratios and electrolyte balance. Poult. Sci. 77:864–869.

Kidd, M. T., E. D. Peebles, S. K. Whitmarsh, J. B. Yeatman, and R.F. Wideman, Jr. 2001. Growth and immunity of broiler chicks as affected by dietary arginine. Poult. Sci. 80:1535–1542.

Kidd, M. T., J. P. Thaxton, J. B. Yeatman, S. J. Barber, and W. S. Virden. 2002. Arginine responses in broilers: live performance. Poult. Sci. 80(Suppl. 1):114.

Kim, S. W., R. D. Mateo, Y. L. Yin, and G. Wu. 2007. Functional amino acids and fatty acids for enhancing production performance of sows and piglets. Asian-aust. J. Anim. 20:295–306.

Kogut, M. H. 2009. Impact of nutrition on the innate immune response to infection in poultry. J. Appl. Poult. Res. 18:111–124.

Kubińska, M., B. Tykałowski, A. Koncicki, and J. Jankowski. 2015. Biochemical and immunological responses of young turkeys to vaccination against Ornithobacterium rhinotraheale and different levels of dietary methionine. Polish J. Vet. Sci. 18:807–816.

Kubińska, M., J. Jankowski, J. Juśkiewicz, K. Ognik, A. Czech, J. Celej, and Z. Zduńczyk. 2016. Growth rate and metabolic parameters in young turkeys fed diets with different dietary inclusion levels of methionine. J. Anim. Feed Sci. 25:250–258.

Kwak, H. R., E. Austicb, and R. Rodney. 2001. Dietary arginine-genotype interactions and immune status. Nutrit. Res. 21:1035–1044.

Kwak, H. R., E. Austic, and R. R. Dietert. 1999. Influence of dietary arginine concentration on lymphoid organ growth in chickens. Poult. Sci. 78:1536–1541.

Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. Animals 3:356–369.

Lee, J. E., R. E. Austic, S. A. Naqi, K. A. Golemboski, and R. R. Dietert. 2002. Dietary arginine intake alters avian leukocyte population distribution during infectious bronchitis challenge. Poult. Sci. 81:793–798.

Lehmann, D., M. Pack, and H. Jeroch. 1997. Effects of dietary threonine in starting, growing, and finishing Turkey toms. Poult. Sci. 76:696–702.

Lind, D. S. 2004. Arginine and cancer. J. Nutr. 134:2837S–2841S.

Mieulet, V., L. J. Yan, C. Choisy, K. Sully, J. Procter, A. Kouroumalis, S. Krywawych, M. Pende, S. C. Ley, C. Moinard, and R. F. Lamb. 2010. TPL-2–mediated activation of MAPK downstream of TLR4 signaling is coupled to arginine availability. Sci. Signal. 3:ra61.

Mirzaaghatabar, F., A. A. Saki, P. Zamani, H. Aliarabi, and H. R. Hemati Matin. 2011. Effect of different levels of diet methionine and metabolisable energy on broiler performance and immune system. Food Agr. Immunol. 22:93–103.

Moore, S., and W. H. Stein. 1954. A modified ninhydrin reagent for photometric determination of amino acids and related compounds. J. Biol. Chem. 211:907–913.

Morris, Jr, S.M. 2010. Arginine: master and commander in innate immune responses. Sci. Signal. 3:pe27.

Murawska, D., M. Kubińska, M. Gesek, Z. Zduńczyk, U. Brzostowska, and J. Jankowski. 2018. The effect of different dietary levels and sources of methionine on the growth performance of turkeys, carcass and meat quality. Ann. Anim. Sci. 18:525–540.

Newsholme, P., L. Brennan, B. Rubi, and P. Maechler. 2005. New insights into amino acid metabolism, beta-cell function and diabetes. Clin. Sci. 108:185–194.

NRC: Research Council National 1994. Nutrient Requirements of Poultry, 9th ed. The National Academies Press, Washington, DC, USA.

Oso, A. O., G. A. Williams, O. O. Oluwatosin, A. M. Bamgbose, A. O. Adebayo, O. V. Olowofeso, A. A. Pirgozliev, S. O. Adegbenjo, J. O. Osho, F. Alabi, H. Li, G. Liu, K. Yao, and W. Xin. 2017. Effect of dietary supplementation with arginine on haematological indices, serum chemistry, carcass yield, gut microflora, and lymphoid organs of growing turkeys. Live. Sci. 198:58–64.

Qureshi, M. A. 2003. Avian macrophage and immune response: an Overview. Poult. Sci. 82:691–698.

Ren, W., J. Yin, M. Wu, G. Liu, G. Yang, Y. Xion, D. Su, L. Wu, T. Li, S. Chen, J. Duan, Y. Yin, and G. Wu. 2014. Serum amino acids profile and the beneficial effects of L-arginine or L-glutamine supplementation in dextran sulfate sodium colitis. PLoS One 9:e88335.

Singh, R., S. Pervin, A. Karimi, S. Cederbaum, and G. Chaudhuri. 2000. Arginase activity in human breast cancer cell lines: N(omega)-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells. Canc. Res. 60:3305–3312.

Stechmiller, J. K., B. Langkamp-Henken, B. Childress, K. A. Herrlinger-Garcia, B. Hudgens, and J. Tian. 2005. Arginine supplementation does not enhance serum nitric oxide levels in elderly nursing home residents with pressure ulcers. Biol. Res. Nurs. 6:289–299.

Stevens, B., M. Godfrey, T. Kaminski, and R. Braith. 2000. High intensity dynamic human muscle performance enhanced by a metabolic intervention. Med. Sci. Sport Excerc. 32:2102–2104.

Tizard, I. R. 2012. Veterinary Immunology, 9th ed. Saunders Publishingm, Philadelphia, PA.

Uni, Z., and P. Ferket. 2003. Enhancment of development of oviparous species by in vivo feeding. U.S. Regular Patent US 6,592,878 B2. Washinghton, DC., US.

Veldkamp, T., R. P. Kwakkel, P. P. Ferket, and P. C. M. Simons. 2005. Effect of ambivalent temperature, arginine-to-lysine ratio, and electrolyte balance on performance, carcass, and blood parameters in commercial male turkeys. Poult. Sci. 79:1608–1616.

Waldroup, P. W., J. A. England, M. T. Kidd, and B. J. Kerr. 1998. Dietary arginine and lysine in large white toms. 1. Increasing arginine: lysine ratios does not improve performance when lysine levels are adequate. Poult. Sci. 77:1364–1370.

Wu, B., H. Cui, X. Peng, J. Fang, W. Cui, and X. Liu. 2012. Effect of methionine deficiency on the thymus and the subsets and proliferation on peripheral blood T cell, and serum IL 2 in broilers. J. Int. Agri. 11:1009–1019.

Wu, G. 2013. Functional amino acids in nutritional and health. Amino Aci. 45:407–411.

Wu, G., and C. J. Meininger. 2000. Arginine nutrition and cardiovascular function. J. Nutr. 130:2626–2629.

Xu, Y. Q., Y. W. Guo, B. L. Shi, S. M. Yan, and X. Y. Guo. 2018. Dietary arginine supplementation enhances the growth performance and immune status of broiler chickens Live. Science 209:8–13.

Zduńczyk, Z., J. Jankowski, M. Kubińska, A. Czech, and J. Juśkiewicz. 2017. The effect of different dietary levels DL-methionine and DL-hydroxy analogue on antioxidant and immunologic status of young turkeys. Archiv. Anim. Nutr. 71: 347–361.