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Effects of dietary surpluses of methionine and lysine on growth performance, blood serum parameters, immune responses, and carcass traits of broilers

Samantha Sigolo [a], Ebrahim Deldar[b], Alireza Seidavi [b], Mehrdad Bouyeh[b], Antonio Gallo [a] and Aldo Prandini [a]

[a]Department of Animal Science, Food and Nutrition (DIANA), Università Cattolica Sacro Cuore, Piacenza, Italy; [b]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

ABSTRACT

We evaluated the effects of dietary surpluses (100%, 110%, and 120% of Ross recommendations) of methionine (Met) and lysine (Lys) on growth performance, blood serum parameters, immune responses, and carcass traits of broilers using a completely randomized design with a 3 × 3 factorial arrangement. Broilers fed a diet containing Met and Lys according to Ross recommendations had maximal growth performance. During the starter period, higher Lys decreased intake of feed, energy, and protein (linear, $P < 0.01$) and weight gain (linear, $P = 0.01$). A diet with 110% Lys decreased feed efficiency (quadratic, $P = 0.03$) during the grower period and decreased the pre-slaughtered body weight (tendency; quadratic, $P = 0.10$), and increased serum uric acid (quadratic, $P = 0.03$). Dietary Lys level had an inverse correlation with serum phosphorus (linear, $P = 0.02$) and immune response against Newcastle disease virus after the first vaccination (linear, $P = 0.03$). There were Met × Lys interaction effects on serum glucose ($P = 0.01$), and relative weights of the rectum ($P = 0.02$) and liver ($P = 0.02$). Taken together, our results indicate that dietary surpluses of Met and Lys provided no additional benefits in broiler performance.

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Amino acids; anatomical parts; antibody response; carcass yield; feed efficiency; poultry

Introduction

Methionine (Met) and lysine (Lys) have many important functions in the metabolism of monogastric animals, and consumption of these functional amino acids improves their health, survival, growth, development, and reproduction (Wu 2013; Jankowski et al. 2014; Wu et al. 2014; Liao et al. 2015).

The supplementation of corn and soybean diets with Met and Lys, the two major limiting amino acids, is therefore a common practice in poultry production. The dietary levels of Met and Lys affect broiler growth, performance, and carcass quality (Corzo et al. 2005; Kidd et al. 2005; Zhai et al. 2016). The addition of high levels of synthetic amino acids, such as Met and Lys, can stimulate insulin secretion (Murray et al. 1998), and this increases the uptake of amino acids and protein synthesis in multiple tissues (Sturkie 1986). Typically, a high dietary density of amino acids leads to increased breast meat due to an increase of lean muscle tissue, rather than collagen, a structural protein (Corzo et al. 2010). Zhai et al. (2012) reported that consumption of Met exceeding the dietary requirements increased breast muscle growth *via* regulation of 6 canonical pathways.

Some studies of broilers have shown that dietary levels of Lys and Met that exceed the recommended amounts may increase body weight (BW) (El-Wahab et al. 2015; Zhai et al. 2016), the rate of BW gain (Si et al. 2001; El-Wahab et al. 2015), feed conversion ratio, breast meat yield (Si et al. 2001; Bouyeh and Gevorgyan 2011; Zhai et al. 2016), abdominal fat deposition, fat content of the breast and thigh muscles, and carcass efficiency (Bouyeh and Gevorgyan 2011). Recent experiments on broilers have also shown that supplemental Met and Lys can stimulate the immune responses of broilers (Mirzaaghatabar et al. 2011; Faluyi et al. 2015; Saleh et al. 2018).

The aim of the present study was to investigate the effects of dietary surpluses of Met and Lys on growth performance, blood serum parameters, immune responses, and carcass traits of broilers.

Materials and methods

Birds and housing

Experiments were performed during August and September 2014. All experimental procedures were reviewed and approved by the Institutional Animal Care and Ethics Committee of Islamic Azad University (Rasht Branch, Rasht, Iran).

All facilities and equipment were disinfected prior to bird placement. Two hundred and seventy male Ross 308 chicks (Aviagen, Newbridge, Scotland, UK) were randomly allocated to 9 dietary treatments (3 replicates per treatment). Each group of 10 birds was raised in a 1 × 1.5 m wire mesh pen. The initial mean and range of BW was similar in each of the pens (42.7 ± 0.8 g). Each cage floor was covered with paper roll litter, and each pen had an automatic drinker and a pan feeder. Water and experimental diets were offered *ad libitum*. Light schedule, temperature, humidity, and general conditions were according to the *Ross Broiler Management Manual* (Ross 2009).

CONTACT Alireza Seidavi alirezaseidavi@iaurasht.ac.ir Department of Animal Science, Islamic Azad University, Rasht Branch, Rasht, Iran

Experimental diets

Broiler responses to different levels of Met and Lys were evaluated from day 1 to 14 (starter period), day 15 to 28 (grower period), and day 29 to 42 (finisher period). A 9-way factorial arrangement was used, in which broilers were fed 3 different levels of Lys and 3 different levels of Met (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007). The diets were formulated to be iso-energetic and iso-nitrogenous, and to meet or exceed the nutritional requirements of broilers suggested by Ross (2007) for the starter, grower, and finisher periods. Thus, the dietary levels of available Lys to meet 100%, 110%, and 120% of Ross 308 specifications were 1.27%, 1.40%, and 1.53% of the diet for the starter period; 1.11%, 1.22%, and 1.33% for the grower period; and 0.98%, 1.07%, and 1.17% for the finisher period. The dietary levels of available Met to meet 100%, 110%, and 120% of Ross 308 specifications were 0.47%, 0.52%, and 0.57% of the diet for the starter period; 0.42%, 0.46%, and 0.50% for the grower period; and 0.38%, 0.42%, and 0.46% for the finisher period. All animals were fed the experimental diets in mash form until day 42. Table 1 shows the ingredients, chemical composition, and metabolizable energy content of the diets for the starter, grower, and finisher periods.

Growth performance

Body weight and feed intake (difference of offered feed and refused feed) were measured weekly. Average daily feed intake (ADFI), average daily weight gain (ADG), gain to feed ratio (G:F), average daily energy intake (ADEI), weight gain to metabolizable energy ratio (G:ME), average daily protein intake (ADPI), and weight gain to protein intake ratio (G:P) were calculated for each replicate within each treatment for the starter period, grower period, finisher period, and whole study period (day 1–42).

Carcass measurements

On the last day of the experiment (day 42), 1 bird per pen was randomly chosen, weighed, and killed by cervical dislocation to evaluate the characteristics of the carcass. The weights of the entire defeathered carcass and of the carcass without head and drumsticks were recorded. Viscera and abdominal fat were then removed, and the carcass yield and the relative weights (percentage of eviscerated carcass) of abdominal fat, anatomical parts (breast, drumsticks, wings, and neck), and gut tracts (duodenum, jejunum, ileum, colon, right cecum, left cecum, and rectum) were calculated. At day 42, broilers used to evaluate immune responses were also killed by cervical dislocation, and the relative weights of organs related to the immune system function (thymus, liver, spleen, and bursa of Fabricius) were calculated according to Shabani et al. (2015).

Blood serum parameters

At day 42, blood samples (1.5 mL) were collected into EDTA tubes from a wing vein. Blood serum was separated by centrifugation (Rotofix 32A centrifuge, Hettich, Germany; 1500 × g for 10 min) and chilled at −18°C until analysis. The levels of uric acid, total cholesterol, triglycerides, very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose, total protein, albumin, globulin, alkaline phosphatase (ALP), calcium, and phosphorus were measured using commercial laboratory kits (Pars Azmoon Co.,Tehran, Iran; Golrokh et al. 2016).

Immune responses

All birds were vaccinated against avian influenza (AI) on day 1, infectious bronchitis (strain H120) on days 1 and 16, Newcastle disease virus (NDV, strain *Viscerotropic velogenic*) on days 8 and 20, and Gumboro disease on days 14 and 23. Vaccines were supplied *via* drinking water after a 3 h period of water removal. One bird per replicate was randomly chosen for collection of blood samples from the brachial vein. Blood serum was incubated at room temperature for 1 h, separated by centrifugation (as above), and chilled at −20°C until analysis. Antibody titres (immunoglobulin G2, IgG2) against NDV (within 10 and 22 days after the first and second vaccination) and AI (within 30 and 40 days after the vaccination) were measured using the hemagglutination-inhibition test (Cunningham 1971).

To evaluate the systemic antibody response (Dibaji et al. 2015), birds at 14 and 21 days (2 per replicate), with weights similar to the replicate average, were vaccinated against sheep red blood cells (SRBC) by subcutaneous administration of SRBC suspension in 5% PBS. Fourteen days later, total Ig against SRBC was determined using a hemagglutination assay in serum 7. In U-bottom microtiter plates, two-fold serial dilutions of heat-inactivated serum (at 56°C) were added to PBS (0.01 mol/L; pH 7.4) for assessment of total antibodies, or to PBS with 1.4% 2-mercaptoethanol for assessment of IgG antibodies. All antibody titres were recorded as $\log_2$ of the highest dilution of serum that agglutinated an equal volume of a 0.5% SRBC suspension in PBS. The IgM titre was determined as the difference between the total titre and the IgG titre.

Statistical analysis

All data were tested for normality using the Shapiro–Wilk test before statistical analysis. Growth performance data were analyzed in the starter, grower, and finisher periods and growth performance, blood serum parameters, immune responses, and carcass traits were measured on day 42. A 3 × 3 factorial arrangement of dietary treatments was used for a completely randomized design, and the GLM procedure of SAS (2003) was employed. The model was:

$$Y_{ijk} = \mu + \text{Met}_i + \text{Lys}_j + (\text{Met} \times \text{Lys})_{ij} + e_{ijk},$$

where Y_{ijk} is the response variable, μ is the overall mean, Met_i is the fixed effect of Met level ($i = 3$), Lys_j is the fixed effect of Lys level ($j = 3$), $(\text{Met} \times \text{Lys})_{ij}$ is the first order interaction, and e_{ijk} is the random residual error.

When an interaction was not significant, the IML procedure of SAS (2003) was used to generate contrast coefficients to evaluate

Table 1. Ingredients, chemical composition, and metabolizable energy of the starter, grower, and finisher diets, and levels of Met and Lys relative to Ross recommendations (Ross 2007).

Treatment	Starter (from 1 to 14 d)			Grower (from 15 to 28 d)			Finisher (from 29 to 42 d)		
	Met 100%[a]			Met 100%[b]			Met 100%[c]		
	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%
Ingredients (%)									
Corn	58.94	59.23	59.52	59.87	60.10	60.38	65.09	65.30	65.56
Soybean meal	34.23	33.81	33.40	32.32	31.97	31.59	27.27	26.97	26.63
Soybean oil	2.33	2.26	2.19	4.00	3.95	3.88	3.99	3.94	3.87
Dicalcium phosphate	1.94	1.95	1.95	1.67	1.68	1.68	1.58	1.57	1.57
Calcium carbonate	1.40	1.40	1.40	1.17	1.17	1.17	1.12	1.13	1.13
Vitamin premix[d]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[d]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium chloride	0.28	0.28	0.28	0.32	0.32	0.32	0.32	0.32	0.32
Sodium bicarbonate	0.10	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.14	0.14	0.15	0.10	0.10	0.105	0.09	0.09	0.09
L-Lysine	0.14	0.33	0.52	–	0.16	0.325	–	0.14	0.28
Calculated chemical composition (% as fed)									
Dry matter	86.33	86.14	85.97	87.02	86.86	86.70	87.09	86.97	86.83
Crude protein	22.00	22.00	22.00	21.00	21.00	21.00	19.00	19.00	19.00
Crude fat	4.91	4.85	4.79	6.60	6.55	6.49	6.74	6.69	6.63
Linoleic acid	2.62	2.59	2.56	3.49	3.47	3.43	3.58	3.55	3.52
Crude fibre	2.63	2.62	2.61	2.58	2.57	2.56	2.50	2.49	2.48
Calcium	1.05	1.05	1.05	0.90	0.90	0.90	0.85	0.85	0.85
Phosphorus	0.74	0.74	0.74	0.68	0.68	0.68	0.64	0.64	0.64
Available phosphorus[e]	0.50	0.50	0.50	0.45	0.45	0.45	0.42	0.42	0.42
Potassium	0.87	0.86	0.86	0.84	0.83	0.82	0.75	0.74	0.74
Chloride	0.24	0.27	0.31	0.23	0.26	0.30	0.23	0.26	0.29
Manganese (mg/kg)	402.69	402.55	402.39	400.41	400.30	400.15	398.08	397.96	397.83
Sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Zinc (mg/kg)	323.24	323.09	322.93	322.20	322.08	321.93	320.36	320.24	320.11
Choline (g/kg)	1.52	1.51	1.50	1.47	1.47	1.46	1.37	1.36	1.35
Folic acid (mg/kg)	2.02	2.00	1.99	1.95	1.94	1.93	1.79	1.78	1.77
Available amino acids[e] (% as fed)									
Arginine	1.42	1.40	1.39	1.35	1.34	1.33	1.20	1.19	1.18
Glycine	0.90	0.89	0.88	0.86	0.85	0.85	0.77	0.77	0.76
Serine	1.07	1.06	1.05	1.02	1.02	1.01	0.92	0.91	0.90
Glycine + Serine	2.31	2.28	2.26	2.21	2.19	2.17	1.96	1.95	1.93
Histidine	0.57	0.57	0.56	0.55	0.55	0.54	0.50	0.50	0.49
Isoleucine	0.90	0.89	0.88	0.86	0.85	0.85	0.77	0.76	0.76
Leucine	1.87	1.86	1.84	1.81	1.80	1.79	1.67	1.66	1.65
Lysine	1.27	1.40	1.53	1.11	1.22	1.33	0.98	1.07	1.17
Methionine	0.47	0.47	0.47	0.42	0.42	0.42	0.38	0.38	0.38
Cystine	0.35	0.35	0.35	0.34	0.34	0.37	0.31	0.31	0.31
Methionine + Cystine	0.83	0.82	0.82	0.76	0.76	0.76	0.70	0.69	0.69
Phenylalanine	1.03	1.02	1.01	0.98	0.98	0.97	0.89	0.88	0.87
Tyrosine	0.84	0.84	0.83	0.81	0.80	0.80	0.73	0.72	0.72
Phenylalanine + Tyrosine	1.87	1.86	1.84	1.79	1.78	1.77	1.61	1.60	1.59
Threonine	0.81	0.80	0.80	0.78	0.77	0.77	0.70	0.69	0.69
Tryptofan	0.29	0.29	0.28	0.28	0.27	0.27	0.24	0.24	0.24
Valine	1.00	0.99	0.98	0.96	0.95	0.94	0.87	0.86	0.85
Metabolizable energy[f] (MJ/kg)	12.67	12.67	12.67	13.19	13.19	13.19	13.40	13.40	13.40

Lys, lysine; Met, methionine.
[a]For the starter period, the Met levels (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007) were 0.47, 0.52, and 0.57% of the diet.
[b]For the grower period, the Met levels (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007) were 0.42, 0.46, and 0.50% of the diet.
[c]For the finisher period, the Met levels (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007) were 0.38, 0.42, and 0.46% of the diet.
[d]Amount per kg: vitamin A, 5,000 IU; vitamin D_3, 500 IU; vitamin E, 3 mg; vitamin K_3, 1.5 mg; vitamin B_2, 1 mg; calcium pantothenate, 4 mg; niacin, 15 mg; vitamin B_6, 13 mg; Cu, 3 mg; Zn, 15 mg; Mn, 20 mg; Fe, 10 mg; K, 0.3 mg.
[e]Metabolic availability (from UFFDA output; Pesti and Miller 1993).
[f]Metabolizable energy was estimated using the Carpenter and Clegg equation (Leeson and Summers 2001).

equally-spaced linear or quadratic responses to Met or Lys level. The normal distribution of residuals of the GLM model for each parameter were analyzed by visual inspection of the Q-Q plot of residuals generated using the PLOTS = DIAGNOSTICS option of PROC GLM (SAS 2003). None of the parameters had non-normal distribution of residuals, so there were no transformations of original variables. A P value of 0.05 or below was considered significant, and a P value between 0.05 and 0.10 was considered to indicate a tendency for significance.

Results

Growth performance, carcass traits and relative organ weights

The effects of different levels of Met and Lys on broiler growth performance are shown in Table 2. The results indicate that 110 and 120% Lys levels decreased the ADFI, ADEI, and ADPI (Lys, $P < 0.01$; linear, $P < 0.01$) and consequently the ADG (Lys, $P = 0.03$; linear, $P = 0.01$) during the starter period. Regardless of the Met level,

Table 2. Growth performance of Ross 308 broilers that received diets with different levels of Met and Lys (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007).

	Met 100%			Met 110%			Met 120%				*P*-Value			Lys contrasts	
Item	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	SEM	Met	Lys	Met × Lys	Linear	Quadratic
ADFI (g)															
d1 to d14	25.91	26.09	24.38	26.78	26.16	25.49	26.38	26.51	25.15	0.388	0.11	<0.01	0.69	<0.01	0.05
d15 to d28	81.92	85.72	83.65	86.70	84.81	80.09	87.81	82.59	82.21	2.626	0.98	0.27	0.38	0.12	0.74
d29 to d42	158.18	156.46	160.54	151.48	160.07	166.66	161.15	157.45	153.68	2.919	0.71	0.37	0.02	–	–
d1 to d42	88.67	89.42	89.53	88.32	90.35	90.85	91.78	88.85	87.01	19.232	1.00	1.00	1.00	0.97	0.99
ADG (g)															
d1 to d14	17.78	17.00	17.50	18.52	17.72	16.49	18.04	16.89	16.18	0.592	0.53	0.03	0.52	0.01	0.61
d15 to d28	48.45	43.09	44.60	47.25	45.80	44.08	46.44	42.57	48.88	2.134	0.95	0.15	0.35	0.39	0.08
d29 to d42	91.51	86.22	84.18	83.08	87.70	93.46	88.32	84.01	80.32	2.539	0.17	0.66	0.01	–	–
d1 to d42	52.58	48.77	48.76	49.62	50.41	51.34	50.94	47.83	48.46	10.117	0.99	0.97	1.00	0.85	0.86
G:F															
d1 to d14	0.69	0.65	0.72	0.69	0.68	0.65	0.68	0.64	0.64	0.027	0.38	0.37	0.39	0.46	0.23
d15 to d28	0.59	0.51	0.53	0.54	0.54	0.55	0.53	0.52	0.59	0.022	0.99	0.06	0.10	0.87	0.03
d29 to d42	0.58	0.55	0.52	0.55	0.55	0.56	0.55	0.53	0.52	0.020	0.52	0.40	0.60	0.19	0.84
d1 to d42	0.62	0.57	0.59	0.60	0.59	0.59	0.59	0.56	0.59	0.025	0.75	0.41	0.92	0.56	0.23
ADEI (kcal)															
d1 to d14	75.79	76.32	71.31	78.34	76.51	74.56	77.15	77.54	73.57	1.135	0.11	<0.01	0.69	<0.01	0.05
d15 to d28	243.72	255.01	248.87	257.95	252.30	238.26	261.25	245.69	244.59	7.813	0.98	0.27	0.38	0.12	0.74
d29 to d42	470.58	465.47	477.62	450.66	476.22	495.81	479.42	468.43	457.19	8.685	0.71	0.37	0.02	–	–
d1 to d42	263.36	265.60	265.93	262.31	268.34	269.54	272.61	263.89	258.45	57.394	1.00	1.00	1.00	0.98	0.99
G:ME (g/kcal)															
d1to d14	0.23	0.22	0.25	0.23	0.23	0.22	0.23	0.22	0.22	0.009	0.38	0.37	0.39	0.46	0.23
d15 to d28	0.20	0.17	0.18	0.18	0.18	0.18	0.18	0.17	0.20	0.007	0.99	0.06	0.10	0.87	0.03
d29 to d42	0.19	0.19	0.18	0.18	0.18	0.19	0.18	0.18	0.18	0.007	0.52	0.40	0.60	0.19	0.84
d1 to d42	0.21	0.19	0.20	0.20	0.20	0.20	0.20	0.19	0.20	0.009	0.77	0.46	0.94	0.58	0.26
ADPI (g)															
d1 to d14	5.31	5.35	5.00	5.49	5.36	5.22	5.40	5.43	5.16	0.080	0.11	<0.01	0.69	<0.01	0.05
d15 to d28	15.98	16.71	16.31	16.91	16.54	15.62	17.12	16.10	16.03	0.512	0.98	0.27	0.38	0.12	0.74
d29 to d42	29.26	28.95	29.70	28.02	29.61	30.83	29.81	29.12	28.43	0.540	0.71	0.37	0.02	–	–
d1 to d42	16.85	17.00	17.00	16.81	17.17	17.22	17.45	16.89	16.54	3.478	1.00	1.00	1.00	0.97	0.99
G:P															
d1 to d14	3.35	3.18	3.52	3.37	3.30	3.16	3.34	3.11	3.14	0.132	0.38	0.37	0.39	0.46	0.23
d15 to d28	3.03	2.59	2.74	2.80	2.77	2.82	2.72	2.65	3.04	0.113	0.99	0.06	0.10	0.87	0.03
d29 to d42	3.13	2.98	2.84	2.97	2.96	3.03	2.97	2.89	2.83	0.108	0.52	0.40	0.60	0.19	0.84
d1 to d42	3.17	2.92	3.03	3.05	3.01	3.00	3.01	2.88	3.00	0.098	0.62	0.24	0.82	0.44	0.13

ADFI, average daily feed intake; ADG, average daily gain; G:F, gain to feed ratio; ADEI, average daily energy intake; G:ME, gain to metabolizable energy intake ratio; ADPI, average daily protein intake; G:*P*, gain to protein intake ratio.
Significance was considered at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

the G:F, G:ME, and G:P tended to be the lowest for the 110% Lys level during the grower period (Lys, $P = 0.06$; quadratic, $P = 0.03$). A Met × Lys interaction effect occurred during the finisher period. In particular, the highest ADFI, ADEI, ADPI ($P = 0.02$), and ADG ($P = 0.01$) occurred for 110% Met and 120% Lys levels. However, from day 1 to 42, we observed no beneficial effects of surplus levels of Met and Lys on broiler growth performance.

The effects of different levels of Met and Lys on carcass traits and relative organ weights are shown in Table 3. The results show that regardless of the Met level, the pre-slaughtered BW tended to be the lowest for the 110% Lys level (Lys, $P = 0.10$; quadratic, $P = 0.10$). A Met × Lys interaction effect influenced the relative weights of the rectum ($P = 0.02$), liver ($P = 0.02$), and bursa of Fabricius (tendency, $P = 0.06$). In particular, the rectum relative weight was greatest for 100% Lys and 110% Met levels. The highest liver relative weight was at 100% Lys and 100% Met levels. A 110% Lys level tended to increase the relative weight of the bursa of Fabricius when Met was at the 100% or 120% levels, but this parameter was lowest at the 110% Met level.

Blood serum parameters

The effects of different levels of Met and Lys on blood serum parameters are shown in Table 4. In particular, a Lys level of 110% increased the uric acid level (Lys, $P = 0.05$; quadratic, $P = 0.03$). HDL cholesterol tended to increase with increasing Met level (Met, $P = 0.09$; linear, $P = 0.04$). A Met × Lys interaction effect influenced glucose (Met × Lys, $P = 0.01$) and ALP (Met × Lys tendency, $P = 0.06$). In particular, for a Met level of 100%, glucose concentration increased as the Lys level increased; however, when Met was at the 110% or 120% levels, the highest glucose concentration occurred for a Lys level of 110%. For a Lys level of 110%, the ALP level tended to be greater for 100% and 120% Met levels. Phosphorus concentration decreased as Lys level increased (Lys, $P = 0.05$; linear, $P = 0.02$).

Immune responses

Analysis of immune responses showed there was a Met × Lys interaction effect (tendency, $P = 0.09$) on the immune response against AI within 40 days after vaccination (Table 5). In particular, the antibody response tended to be lowest for a Lys level of 110% and a Met level of 100%, and highest for a Lys level of 110% and a Met level of 120%. However, the immune response against AI within 40 days after injection tended to decrease for a Lys level of 120% and Met levels of 110% and 120%. The antibody response against NDV after the first vaccination tended to increase with increasing Lys level (Lys, $P = 0.09$; linear, $P = 0.03$).

Table 3. Carcass traits and relative weights (percentage of eviscerated carcass) of abdominal fat, anatomical parts, gut tracts, and organs related to immune system function of Ross 308 broilers that received diets with different levels of Met and Lys (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007).

	Met 100%			Met 110%			Met 120%				*P*-Value			Met contrasts		Lys contrasts	
Item	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	SEM	Met	Lys	Met × Lys	Linear	Quadratic	Linear	Quadratic
Carcass traits																	
Pre-slaughtered BW (g)	3,323	2,639	2,851	2,748	2,741	2,714	2,797	2,704	2,783	143.5	0.20	0.10	0.18	0.15	0.27	0.16	0.10
Carcass weight[a] (g)	2,583	2,273	2,443	2,336	2,360	2,339	2,428	2,301	2,462	96.4	0.54	0.22	0.51	0.66	0.32	0.67	0.09
Full carcass weight[b] (g)	2,406	2,109	2,267	2,170	2,185	2,172	2,258	2,131	2,286	92.2	0.54	0.20	0.53	0.64	0.32	0.64	0.09
Eviscerated carcass weight (g)	1,831	1,664	1,714	1,622	1,688	1,697	1,767	1,607	1,735	75.8	0.57	0.37	0.50	0.60	0.36	0.69	0.18
Carcass yield (%)	76.10	78.91	75.68	74.70	77.31	78.16	78.26	75.38	75.88	1.493	0.95	0.77	0.17	0.75	0.99	0.86	0.49
Abdominal fat and anatomical parts (%)																	
Abdominal fat	1.58	1.70	1.76	2.17	1.93	2.06	1.88	1.88	1.76	0.285	0.29	0.98	0.95	0.50	0.16	0.95	0.87
Breast	33.82	35.16	34.39	32.20	34.66	35.56	33.10	32.75	33.69	1.320	0.48	0.55	0.86	0.25	0.71	0.45	0.44
Drumsticks	29.88	30.56	29.50	29.62	29.33	31.73	31.82	29.76	30.75	0.821	0.49	0.50	0.52	0.25	0.79	0.74	0.26
Wings	3.37	3.36	3.15	3.14	3.44	3.15	3.35	3.21	3.32	0.114	0.74	0.47	0.30	0.84	0.47	0.48	0.32
Neck	2.32	2.46	2.48	2.12	2.32	2.24	2.54	2.46	2.39	0.154	0.17	0.78	0.82	0.76	0.07	0.72	0.55
Gut (%)																	
Duodenum	0.75	0.78	0.79	0.80	0.83	0.70	0.73	0.66	0.67	0.056	0.11	0.61	0.57	0.08	0.25	0.37	0.70
Jejunum	2.19	1.51	1.94	2.51	1.73	2.02	1.72	2.51	2.05	0.327	0.68	0.70	0.17	0.43	0.68	0.61	0.51
Ileum	2.04	1.60	1.97	2.44	1.88	1.90	1.89	2.18	1.88	0.295	0.70	0.56	0.56	0.64	0.49	0.39	0.53
Colon	0.20	0.15	0.25	0.19	0.13	0.23	0.14	0.16	0.20	0.046	0.42	0.43	0.44	0.88	0.20	0.36	0.36
Right cecum	0.25	0.41	0.25	0.32	0.36	0.26	0.26	0.26	0.31	0.045	0.64	0.11	0.92	0.35	0.95	0.17	0.10
Left cecum	0.28	0.31	0.20	0.25	0.31	0.31	0.24	0.24	0.29	0.047	0.70	0.73	0.42	0.82	0.43	0.77	0.47
Rectum	0.05	0.06	0.06	0.15	0.07	0.06	0.05	0.06	0.07	0.016	0.04	0.21	0.02	0.76	0.01	0.13	0.35
Organs related to immune system (%)																	
Thymus	0.31	0.28	0.23	0.28	0.18	0.26	0.26	0.31	0.21	0.046	0.70	0.43	0.32	0.69	0.47	0.20	0.94
Liver	3.73	2.35	2.76	2.81	2.87	2.61	2.86	3.11	2.82	0.220	0.55	0.08	0.02	0.92	0.28	0.04	0.34
Spleen	0.08	0.11	0.10	0.13	0.12	0.09	0.09	0.11	0.08	0.021	0.57	0.40	0.70	0.64	0.34	0.59	0.22
Bursa of Fabricius	0.14	0.26	0.15	0.17	0.12	0.15	0.12	0.18	0.15	0.028	0.22	0.19	0.06	0.18	0.26	0.85	0.07

[a]Defeathered carcass weight.
[b]Full carcass without head and drumsticks.
Significance was considered at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

Table 4. Blood serum parameters of Ross 308 broilers that received diets with different levels of Met and Lys (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007).

	Met 100%			Met 110%			Met 120%				*P*-Value			Met contrasts		Lys contrasts	
Item	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	SEM	Met	Lys	Met × Lys	Linear	Quadratic	Linear	Quadratic
Uric acid (mg/dl)	5.00	5.13	5.07	4.00	5.87	4.60	4.83	6.13	5.77	0.500	0.20	0.05	0.48	0.23	0.17	0.21	0.03
Total cholesterol (mg/dl)	137.00	123.67	130.33	106.00	144.33	144.67	147.33	138.00	146.00	14.733	0.48	0.70	0.38	0.28	0.61	0.41	0.99
Triglycerides (mg/dl)	85.33	126.00	108.33	108.67	114.00	119.33	111.00	124.33	111.33	18.177	0.81	0.43	0.87	0.55	0.82	0.46	0.29
VLDL (mg/dl)	17.00	25.33	21.67	21.67	23.00	24.00	22.00	25.00	22.33	3.643	0.81	0.38	0.87	0.56	0.80	0.42	0.26
HDL cholesterol (mg/dl)	66.67	70.00	65.33	81.67	80.33	80.67	71.33	88.33	89.33	8.827	0.09	0.65	0.75	0.04	0.37	0.48	0.37
LDL cholesterol (mg/dl)	53.33	28.33	43.33	36.00	41.00	40.00	54.00	39.33	34.33	9.973	0.90	0.36	0.53	0.91	0.66	0.31	0.32
LDL/HDL	0.85	0.42	0.67	0.44	0.52	0.52	0.89	0.48	0.43	0.200	0.64	0.31	0.57	0.77	0.38	0.28	0.27
Glucose (mg/dl)	213.00	241.00	258.33	232.00	241.00	235.00	247.00	254.33	231.33	6.934	0.32	0.05	0.01	0.25	0.34	0.07	0.07
Total protein (g/dl)	3.77	3.17	3.33	3.67	3.47	3.67	3.60	3.50	3.60	0.249	0.66	0.36	0.83	0.49	0.56	0.49	0.21
Albumin (g/dl)	1.43	1.23	1.27	1.43	1.37	1.47	1.33	1.27	1.47	0.132	0.59	0.51	0.84	0.68	0.35	1.00	0.25
Globulin (g/dl)	2.33	1.93	2.07	2.23	2.10	2.20	2.27	2.23	2.13	0.136	0.67	0.24	0.68	0.38	0.86	0.21	0.24
ALP (U/l)	314.33	518.67	298.00	365.67	233.17	377.63	286.20	689.67	395.07	90.671	0.23	0.10	0.06	0.29	0.17	0.64	0.04
Calcium (g/dl)	10.33	10.43	10.67	10.67	10.57	10.77	10.53	10.53	10.63	0.220	0.59	0.53	0.98	0.63	0.37	0.34	0.58
Phosphorus (g/dl)	7.07	7.63	6.87	8.13	7.17	7.50	7.87	7.43	6.87	0.283	0.23	0.05	0.11	0.40	0.14	0.02	0.89

VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALP, alkaline phosphatase.
Significance was considered at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

Table 5. Antibody titres against avian influenza (AI), Newcastle disease virus (NDV), and sheep red blood cells (SRBC) in Ross 308 broilers that received diets with different levels of Met and Lys (100%, 110%, and 120% of Ross 308 recommendations; Ross 2007).

Item	Met 100%			Met 110%			Met 120%			SEM	*P*-Value			Met contrasts		Lys contrasts	
	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%	Lys 100%	Lys 110%	Lys 120%		Met	Lys	Met × Lys	Linear	Quadratic	Linear	Quadratic
AI, IgG2																	
Within 30d	2.33	2.33	2.67	2.67	3.00	2.33	2.00	4.00	2.00	0.685	0.90	0.30	0.47	0.70	0.82	1.00	0.13
Within 40d	4.67	3.33	4.00	3.67	3.67	3.00	3.33	4.67	3.00	0.471	0.37	0.27	0.09	0.40	0.26	0.17	0.42
NDV, IgG2																	
10d after 1st injection	1.33	2.00	2.33	2.00	1.67	2.33	1.33	2.00	2.67	0.471	0.95	0.09	0.76	0.78	0.87	0.03	0.74
22d after 2nd injection	5.33	4.00	5.00	3.67	6.33	5.00	4.00	6.67	5.33	0.786	0.69	0.14	0.11	0.40	0.92	0.24	0.11
SRBC																	
14d after 1st injection																	
Total Ig	1.00	1.00	0.33	0.33	1.33	0.33	1.33	1.00	0.33	0.509	0.87	0.18	0.74	0.79	0.65	0.20	0.18
IgG	0.33	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.157	0.61	0.16	0.74	1.00	0.33	0.10	0.33
IgM	0.67	1.00	0.33	0.33	1.33	0.33	1.00	1.00	0.33	0.430	0.94	0.11	0.83	0.76	0.86	0.36	0.06
14 after 2nd injection																	
Total Ig	2.33	3.33	1.66	3.33	2.00	2.33	2.67	2.66	2.33	0.762	0.98	0.53	0.59	0.86	0.92	0.30	0.68
IgG	0.33	1.67	0.33	1.67	0.67	1.00	1.00	0.66	0.00	0.430	0.31	0.22	0.11	0.54	0.16	0.13	0.37
IgM	2.00	1.66	1.33	1.66	1.33	1.33	1.67	2.00	2.33	0.521	0.44	0.96	0.77	0.44	0.31	0.80	0.88

Significance was considered at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

Discussion

Growth performance, carcass traits and relative organ weights

Our results showed that the dietary recommendations of Ross (2007) for Met and Lys led to maximum growth performance, in terms of ADFI, ADG, G:F, ADEI, G:ME, ADPI, and G:P. During the starter and grower periods and throughout the study, Met surpluses of 10% and 20% did not affect broiler growth performance. During the finisher period, a 10% Met surplus and a 20% Lys surplus increased ADFI, ADEI, ADPI, and ADG. During the starter period, excess Lys negatively affected feed, energy, and protein intake and consequently, led to less weight gain. A 10% Lys surplus tended to decrease feed efficiency (G:F, G.ME, and G:P) during the grower period. Thus, regardless of the Met level, a 10% Lys surplus tended to decrease the broiler pre-slaughtered BW. These findings are in agreement with Cengiz et al. (2008), who reported that a Met surplus had no effect on broiler growth performance, but that a Lys surplus decreased the pre-slaughtered BW.

Thus, our results show that diets with increased levels of free Met and Lys do not improve broiler growth performance, even though free synthetic amino acids should have higher availability than amino acids in intact proteins (Ciftci and Ceylan 2004; Abbasi et al. 2014; Sigolo et al. 2017).

In contrast to our findings, Bouyeh and Gevorgyan (2011) showed that broiler diets with supplemental Met and Lys improved the feed conversion ratio. El-Wahab et al. (2015) reported increased BW when diets had additional Met and Lys. However, the literature has conflicting reports regarding the effects of Met and/or Lys excesses on broiler growth performance. Some of these discrepancies might be due to the use of different experimental conditions, such as Met and Lys levels and experiment duration.

In this study, Met and Lys surpluses had no effect on carcass traits (except for pre-slaughtered BW, as discussed above), abdominal fat, and yield of anatomical parts. The highest relative weight of the rectum was achieved by a 110% Met and 100% Lys diet. Bouyeh and Gevorgyan (2011) found that the relative weight of the broiler liver increased linearly with the dietary levels of Lys and Met. In contrast, we found that broilers fed the standard diet had livers with the highest relative weights. The bursa of Fabricius is the primary lymphoid organ responsible for the establishment and maintenance of the B cell compartment in birds (Jankowski et al. 2014). Wu et al. (2013) reported that a Met deficiency restrained the development of bursa of Fabricius, and thereby reduced the humoral immunity of chickens. However, Bouyeh (2012) found that supplemental Met and Lys did not affect the relative weight of this organ. Our results indicated that a 10% Lys surplus, without additional Met or with a 20% Met surplus, tended to produce the highest relative weights of bursa of Fabricius.

Blood serum parameters

In agreement with growth performance results, the blood serum parameters indicated a status of excess or imbalanced dietary amino acid profile when 10% Lys surplus was included in broiler diets. In fact, a 10% Lys surplus, and independently from the Met level, led to the highest concentration of uric acid. Broilers produce uric acid as the main end product of nitrogen metabolism (Hosseintabar et al. 2015). In particular, birds metabolize excess or imbalanced dietary amino acids into various C-compounds and ammonia, and then convert the ammonia (which is highly toxic) into uric acid (Namroud et al. 2008; Karami et al. 2018). As precursors of L-carnitine, Lys and Met can play important roles in lipid and energy metabolism (Borum 1983). L-carnitine has hypolipidemic effects, in that it reduces circulating concentrations of cholesterol, triglycerides, free fatty acids, phospholipids, and VLDL, and it increases the concentrations of HDL, intermediate density lipoprotein (IDL), and LDL (Diaz et al. 2000; Arslan 2006). Chickens experience significant increases in plasma cholesterol, LDL, and HDL when there are dietary surpluses of Met and Lys (Bouyeh and Gevorgyan 2011). Our results showed that an increased Met level tended to increase the HDL concentration. L-carnitine also has a gluconeogenic effect (Arslan et al. 2004; Arslan 2006). In agreement, we found that the highest blood glucose concentrations were in broilers that received a 20% Lys surplus when there was no Met surplus, and a 10% Lys surplus when there was a 20% Met surplus. In accordance with our findings, Bouyeh and Gevorgyan (2011) found that blood glucose increased in birds fed supplemental Lys and Met.

ALP activity is a standard indicator of liver status, and a significantly increased level indicates liver damage (Arslan et al. 2003; Saeid et al. 2014). ALP also plays an important role in bone mineralization. Previous research indicated that serum ALP activity is inversely proportional to the dietary phosphorus level (and thus serum phosphorus concentration), suggesting that ALP synthesis depends on phosphorus level (Bilal et al. 2015). We found that blood serum phosphorus concentration decreased as Lys level increased, indicating increased phosphorus utilization. Nevertheless, we found the highest blood serum ALP levels in broilers that received a 10% Lys surplus without supplemental Met or with a 20% Met surplus.

Immune responses

Both Met and Lys function in antibody synthesis, so adequate dietary levels are needed for immune system function (Bouyeh 2012). Moreover, Met may have direct or indirect roles in specific immune system responses; it is a precursor of glutathione, which protects cells from oxidative stress, and is required for the synthesis of polyamines (e.g. spermine and spermidine), which function in nuclear and cell division processes (Bouyeh 2012). Previous studies showed that supplemental Met and/or Lys can increase the level of antibodies against NDV in broilers (Mirzaaghatabar et al. 2011; Bouyeh 2012; Faluyi et al. 2015). Similarly, we found that birds fed surplus Lys tended to have enhanced immune responses against NDV after the first vaccination. However, the dietary levels of Met and Lys needed for optimal immunostimulatory activity remain unknown. Our measurements showed that Met and Lys surpluses did not affect immune responses against AI within 30 days after vaccination, responses against NDV after the second injection, and responses against SRBC. However, when broiler diets had no Met and Lys surpluses, the highest IgG2 level against AI occurred within 40 days after injection.

Conclusion

Our study of broilers showed that optimal growth performance, blood serum parameters, immune responses, and carcass traits were achieved when the dietary levels of Lys and Met followed the Ross recommendations. Supplemental Met and Lys provided no additional benefits.

Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Samantha Sigolo http://orcid.org/0000-0001-9081-0565
Alireza Seidavi http://orcid.org/0000-0002-1903-2753
Antonio Gallo http://orcid.org/0000-0002-4700-4450
Aldo Prandini http://orcid.org/0000-0002-8650-8766

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