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Original article

The effect of dietary L-arginine intake on the level of antibody titer, the relative organ weight and colon motility in broilers

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Abstract

This study was carried out to determine the effect of L-arginine (L-Arg) levels in diet at the starter, grower and finisher phases on immune response, organ development, nitric oxide (NO) metabolism and colon motility in broilers. A total of 500 one-day-old Ross-308 broiler chickens of mixed sex were separated into one Arg-deficient group and four experimental groups. Each group was then divided into five subgroups of 20 birds each. Arginine deficient group for all phases was fed a basal diet which contained 10% less L-Arg than optimum Arg requirement recommended by the breeder. Experimental groups were fed a basal diet supplemented with L-Arg which was progressively 10% increased in groups. Thus, the diet contained 90, 100, 110, 120 and 130% of optimum Arg requirement for each phases in groups, respectively.

The highest serum infectious bursal disease antibody titer (IBD) was observed in the experimental group which was fed the diet containing 110% L-Arg at grower phase ($P<0.05$), whereas Newcastle disease antibody titer did not differ between groups. The relative weight of spleen increased in groups which were fed the diet containing 120 and 130% L-Arg at starter phase as compared to Arg-deficient group ($P<0.05$). The group which was fed the diet containing 110% L-Arg showed highest relative weight of bursa Fabricii at grower ($P<0.05$) and finisher ($P<0.01$) phases. It was observed that serum nitric oxide (NO) concentration decreased in Arg-deficient group ($P<0.05$). The amplitude of spontaneous colon contractility did not differ between groups at the end of all three phases. However, the frequency of spontaneous colon contractility in the Arg deficient group was higher at starter ($P<0.05$), grower ($P<0.01$) and finisher ($P<0.05$) phases.

These results suggest that the supplementation of L-Arg at higher level than optimum Arg requirement in broiler diet has minimal effect on parameters investigated in the study. However, L-Arg-deficiency may negatively affect immune response and the motility of gastrointestinal system due to disruption of NO metabolism at three phases.

Key words: broiler, L-arginine, immune response, organ development, nitric oxide, colon motility

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Introduction

Moderate to severe nutritional deficiencies of essential amino acids affect immune responses by depressing antibody titers, reducing lymphocyte populations, lowering component titres, and increasing susceptibility to infections (Konashi et al. 2000). Arginine is an essential dibasic amino acid for adult chickens as well as growing animals (Lee et al. 2002). It has beneficial effects on immune function of poultry (Kwak et al. 1999, Lee et al. 2002). Arginine is also involved in wound healing (Evoy et al. 1998) and improves immunity against some avian parasites (Allen and Fetterer 2000). Studies show that immunity is supported by the addition of Arg to the diet due to the enhanced release of nitric oxide (NO) from macrophages (Sung et al. 1991, Webel et al. 1998), the weight of lymphoid organs (Kwak et al. 1999), and the ratio of heterophils/lymphocytes (Su and Austic 1998; Lee et al. 2002). However, the addition of too much Arg to the diet of broiler chicks is not associated with any significant changes in the weight of lymphoid organs or plasma antibody concentrations of those vaccinated against Newcastle disease virus (ND) (Kidd et al. 2001). Nitric oxide synthesized from Arg, an endogenous donor of NO, by nitric oxide synthase (NOS) enzyme shows toxic effect for microorganism by binding some enzymes in bacterial cell wall which are critical for the life cycle of the microorganism (Rodeberg et al. 1995). The infection capability of some microorganisms such as *Mycobacterium avium* is related to resistance of bacteria to NO (McCall et al. 1991).

In mammals, NO secreted from gastro-intestinal system (GIS) influences the motility (Shah et al. 2001), blood flow (Bult et al. 1990, Boeckxstaens et al. 1991), absorption of electrolyte and water (Izzo et al. 1998), mucosal preservation and inflammation process (Wright et al. 1992). Nitric oxide released from non-adrenergic non-cholinergic (NANC) nerves in intestines decreases intestinal motility in rat (Lie and Rand 1990), mice (Bani et al. 2002) and dog (Bult et al. 1990), *in vitro*. Similarly, the diet supplemented with sodium nitroprusside (SNP), an exogenous NO donor increases the nNOS expression in jejunum of broilers, while *N*-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, decreases the nNOS expression (Bulbul et al. 2013).

Previous studies in broiler chickens demonstrated the effect of adequate or higher Arg levels described in National Research Council (NRC) on growth performance and immunity (Kwak et al. 1999, Corzo et al. 2003**,** Cengiz and Kuçukersan 2010). However, to the authors; knowledge, there are no previous studies performed to investigate the effect of diet formulated with decreased and progressively increased Arg on growth performance during three time periods, as well as on NO metabolism and large intestinal motility. Therefore, the objectives of the present study were to evaluate the effects of L-Arg supplemented at different levels to diet on the immune response, organ development, NO metabolism and colon motility during the starter, grower and finisher phases in broilers.

Materials and Methods

Animals, experimental designs and diets

This study was carried out at the Animal Research Center of Afyon Kocatepe University, Turkey, after approval by the local ethical committee.

Five hundred, one-day old, chickens of mixed sex (Ross-308) were obtained from a commercial hatchery, weighed and randomly separated into five groups as follows: Arginine-deficient group and four experimental groups. Each group was then divided into five subgroups that consisted of 20 birds each. Experiments were carried out for 42 days. Feed and water were provided *ad libitum* and the daily lighting regimen was 23 hours of light and 1 hour of dark throughout the study.

A vaccination program against infectious bursal disease (IBD), Newcastle disease (ND) and infectious bronchitis (IB) is shown in Table 1 The chicks were reared on the floor of pens (2x1 m) in a curtain-sided broiler house. Pine wood shavings were used as litter material. The birds were housed at 12 birds /m^2. The temperature was 34 ± 1°C during the first week of the study and was gradually reduced to 26 ± 1°C by the third week. Thereafter, the study was maintained at a room temperature of 24°C.

Dietary responses to L-Arg (Sigma A5131 powder) were evaluated for starter phase (0-10 days), grower phase (11-28 days) and finisher phase (29-42 days). Arginine-deficient group for all phases was fed the basal diet which contained 10% less L-Arg than optimum Arg requirement recommended by the breeder. Thus, the diet was corresponded to 90% L-Arg of optimum Arg requirement. Experimental groups were fed basal diet which included progressive increments of 10% L-Arg thereby; Arg requirement was achieved with the rate of 100, 110, 120 and 130% in groups. The Arg-deficient basal diets contained 13.5, 11.4 and 9.9 g/kg Arg, 221.4, 215.7 and 196.5 g/kg crude protein (CP) and 3023, 3159 and 3173 kcal/kg metabolisable energy (ME) in each of three phases, respectively. All nutrients met or exceeded the nutrient requirements for broiler chickens (Broiler Nutrition Specifications 2007). The levels of CP and Arg of

Table 1. The vaccination schedule used in the study.

IBD	ND	IB	The day of vaccination
Inactive, sc	Inactive, sc	–	Day 0
	Active, dw	Active, dw	Day 7
Active, dw			Day 14
	Active, dw		Day 21

Sc: Subcutan, dw: Drink water

Table 2. Composition of the Arg-deficient basal diets (g/kg).

	Phases		
Ingredients	Starter	Grower	Finisher
Corn	535.3	553.8	572.2
Corn gluten meal	66.0	90.0	90.0
Boncalite	–	–	50.0
Soybean meal	147.5	32.0	4.3
Full fat soybean	171.4	240.0	196.3
Meat bone meal	40.0	40.0	45.0
Vegetable oil	15.0	20.0	25.0
Calcium carbonate	6.4	6.0	2.8
Dicalcium phosphate	6.2	5.5	2.5
Salt	1.9	2.0	2.0
DL-Methionine	2.2	2.0	0.7
L-Lysine HCl	3.1	3.7	2.7
Sodium bicarbonate	2.0	2.0	2.0
Vitamin premix*	2.0	2.0	3.0
Mineral premix**	1.0	1.0	1.5
Calculated composition, g/kg			
ME, kcal/kg	3023	3159	3173
Crude protein	222.0	208.0	190.0
Calcium	9.7	9.2	8.0
Available phosphorus	4.6	4.4	4.2
Methionine + Cystine	9.9	9.6	8.0
Lysine	13.1	12.4	10.4
Arginine	13.8	12.1	10.1
Analyzed composition, g/kg			
Crude protein	221.4	215.7	196.5
Arginine	13.5	11.4	9.9

* Vitamin premix provided per 2.5 kilogram of diets: 12 000 000 IU vitamin A, 2 500 000 IU vitamin D3, 40 000 mg vitamin E, 5 000 mg vitamin K3, 3 000 mg vitamin B1, 6 000 mg vitamin B2, 5 000 mg vitamin B6, 20 mg vitamin B12, 25 000 mg niacin, 12 000 mg pentatonic acid, 1 000 mg folic acid, 50 mg biotin, 10 000 mg BHT

** Mineral premix provided per 2,5 kilogram of diets: 100 000 mg calcium, 100 000 mg magnesium, 70 000 mg manganese, 150 mg cobalt, 400 mg iota, 150 mg selenium, 25 000 mg ferric, 5 000 mg cupper, 60 000 mg ZnO.

the basal diets, including corn, corn gluten, soybean meal, full-fat soybean and meat and bone meal, were analysed before being used in formulation. The CP levels in the diets and raw feed materials was analysed using methods of AOAC (2000). Arginine levels in the diet were determined by LC-MS-MS (Applied Biosystems API-3200) in a laboratory (ANT Technical Devices Laboratories, Bursa) (Table 2).

Determination of IBD and ND antibody titers and heterophil/lymphocyte ratio

Blood samples from 10 birds randomly selected from groups (two birds/replicate) were taken from wing veins to sterile tubes with anti-coagulant (heparine) without anti-coagulant and at the end of starter, grower and finisher phases. After clotting at room temperature for 1 hour and centrifugation (3000 rpm, 15 munites) serum was carefully harvested.

The anti-IBD antibody titer was determined by ELISA with a commercial test kit (IBD ELISA kit, Bio-check Company, Holland) according to the manufacturer's instructions, in an ELISA reader. Newcastle disease virus was determined by the haemagglutination inhibition method. The haemagglutinin titers were expressed as the log2 of the highest dilution showing visible agglutination. Blood smear for each broiler was prepared and fixed with methanol. Then, the smears were stained immediately with Giemsa 100% and rinsed with distilled water and air- dried. Heterophil/lymphocyte (H/L) ratio was counted from 100 cells per slide and classified using oil immersion microscopy at 100× (Dumonceaux and Harrison 1994).

Relative organ weights

At the end of each three phases, 20 birds from each group (4 birds from each replicate) were randomly selected. The broilers were slaughtered by severing the jugular vein. The lymphatic organs (bursa of Fabricius and spleen) and liver were immediately removed, carefully stripped of adhering connective tissue and individually weighed. Relative organ weights were calculated as percentage of body weight.

Nitric oxide concentration

Serum NO concentrations were determined according to the procedure of Miranda et al. (2001). Nitrate was reduced to nitrite with vanadium (III) and then nitrite level measured by using Griess reagents. Serial dilutions 0.5-200 μM of Sodium nitrate (Merck, Germany) were used as standards. The results were expressed as μM.

The preparation of isolated smooth muscle strips of mid colon for contractility experiments

The mid colon samples were collected about 15 min after exsanguinations and transported on ice to the laboratory within 30 min. Then, samples were put into a dissecting Petri dish containing Krebs' solution (KS: NaCl 118 mmol/l KCl 4.7 mmol/l, $CaCl_2$ 2.5 mmol/l, $MgSO_4$ 1 mmol/l, KH_2PO_4 1 mmol/l, glucose 11 mmol/l, $NaHCO_3$ 25 mmol/l), which was continuously ventilated with a gas mixture (95% O_2 and 5% CO_2). Five mm-long ring strips of samples were dissected from the middle part of related tissue and incised longitudinally. Thereafter, longitudinal smooth muscle strips were carefully isolated and one edge of each tissue preparation was fixed to platinum ring electrodes. The opposite edge of the tissue was connected to a force-displacement transducer (model 10-A; MAY, Commat, Ankara, Turkey). Isolated strips were placed in a four chambers organ baths (IOBS 99 Isolated Tissue Bath Stand Set, Commat) filled with 20 ml, which were continuously oxygenated (95% O_2 and 5% CO_2) at 37°C. The isometric smooth muscle activity of mid colon were monitored and recorded by computer via the force transducer and an acquisition system (model MP30 WSW with Biopac Student Lab, PRO Software, Biopac Systems, Commat).

Recording of isometric mid colon contractility

The mid colon in organ baths were kept in KS for at least 1 h before the recordings to enable the tissues to adapt to the environment and the solution was refreshed at 15 min intervals. The appropriate resting tension for the strips was determined in initial experiments. The strips were placed under progressive increments of tension. Optimal tension relationships were achieved with resting tensions of 2 g for the strips. Therefore, a resting tension of 2 g was applied to the tissues. After the completion of the 30 min baseline period, contractions of longitudinal strips of mid colon for each animal were visualized and recorded to determine normal spontaneous contractions (Bulbul et al. 2007).

Statistics

Data were analyzed under a completely randomized design using the General Linear Models

Table 3. Effect of different L-Arg levels on serum IBD (mean titre) and ND (log 2 titre) antibody titers and H/L ratio in broiler during the starter, grower and finisher phases.

	Treatment[1]					SEM	P<	
	90	100	110	120	130		Linear	Quadratic
IBD								
Starter phase	1403	2861	4242	3094	2886	335.5	NS	NS
Grower phase	6635^{b}	7298^{b}	8201^{a}	6161^{b}	5753^{b}	259.4	NS	0.05
Finisher phase	10028	10857	9646	10845	9368	377.5	NS	NS
ND								
Starter phase	7.00	6.50	6.85	7.42	7.00	0.14	NS	NS
Grower phase	7.37	7.87	8.14	8.12	7.87	0.16	NS	NS
Finisher phase	9.85	9.25	10.1	9.25	9.75	0.17	NS	NS
H/L								NS
Starter phase	0.38	0.39	0.33	0.38	0.34	0.08	NS	NS
Grower phase	0.43	0.41	0.38	0.42	0.43	0.01	NS	NS
Finisher phase	0.43	0.45	0.44	0.45	0.46	0.01	NS	NS

[1] These values accomplished 90, 100, 110, 120 and 130% of L-Arg requirements for each phases in Arg-deficient group and experimental groups, respectively.
Letters (a, b) in the same line indicate significant differences between different letters.
NS: Not significant

Table 4. Effect of different L-Arg levels on the relative organ weights (%) in broilers during the starter, grower and finisher phases.

	Treatment[1]					SEM	P<	
	90	100	110	120	130		Linear	Quadratic
Spleen								
Starter phase	0.05^{b}	0.06^{ab}	0.06^{ab}	0.07^{a}	0.07^{a}	0.001	0.05	NS
Grower phase	0.13	0.13	0.15	0.16	0.15	0.07	NS	NS
Finisher phase	0.20	0.20	0.19	0.16	0.18	0.01	NS	NS
Bursa of fabricius								
Starter phase	0.24	0.25	0.28	0.27	0.26	0.001	NS	NS
Grower phase	0.08^{c}	0.08^{bc}	0.12^{a}	0.10^{abc}	0.11^{ab}	0.001	NS	0.05
Finisher phase	0.06^{c}	0.06^{c}	0.10^{a}	0.09^{ab}	0.06^{c}	0.001	NS	0.01
Liver								
Starter phase	3.51	3.59	3.60	3.84	3.78	0.04	NS	NS
Grower phase	2.45	2.78	2.64	2.93	2.74	0.05	NS	NS
Finisher phase	2.39	2.47	2.54	2.57	2.40	0.05	NS	NS

[1] These values accomplished 90, 100, 110, 120 and 130% of L-Arg requirements for each phases in Arg-deficient group and experimental groups, respectively.
Letters (a, b, c) in the same line indicate significant differences between different letters.
NS: Not significant

procedure of the SPSS for Windows. When differences ($P<0.05$) between means were found, means were separated using Tukey's Studentized range test. Linear and Quadratic Arg dose response curves were plotted using the GLM procedure of SPSS. Pen was the experimental unit for all analysis.

Results

The effect of different levels of L-Arg supplementation to the diet given in all phases on antibody titers of IBD and ND, H/L ratio, relative organ weights the amplitude and frequency of spontaneous

Table 5. Effect of different L-Arg levels on the amplitude (g) of spontaneous colon contractility during the starter, grower and finisher phases.

	Treatment[1]					P<
	90	100	110	120	130	
Starter phase	1.12 ± 0.26	1.37 ± 0.20	1.36 ± 0.32	1.27 ± 0.27	1.79 ± 0.28	NS
Grower phase	4.18 ± 0.18	4.66 ± 0.29	4.86 ± 0.54	4.37 ± 0.22	4.92 ± 0.42	NS
Finisher phase	5.80 ± 0.43	6.05 ± 0.35	6.92 ± 0.31	6.74 ± 0.52	7.33 ± 0.23	NS

[1] These values accomplished 90, 100, 110, 120 and 130% of L-Arg requirements for each phases in Arg-deficient group and experimental groups, respectively.
NS: Not significant

Table 6. Effect of different L-Arg levels on the frequency of spontaneous colon contractility (cycle/minute) during the starter, grower and finisher phases (Mean ± SE).

	Treatment[1]					P<
	90	100	110	120	130	
Starter phase	3.70 ± 0.11^{a}	2.98 ± 0.17^{b}	2.88 ± 0.32^{b}	2.73 ± 0.29^{b}	2.60 ± 0.31^{b}	0.05
Grower phase	2.50 ± 0.17^{a}	2.00 ± 0.11^{b}	1.96 ± 0.08^{b}	1.78 ± 0.07^{b}	1.76 ± 0.11^{b}	0.01
Finisher phase	1.93 ± 0.08^{a}	1.86 ± 0.06^{ab}	1.48 ± 0.13^{c}	1.57 ± 0.11^{bc}	1.65 ± 0.10^{abc}	0.05

[1] These values accomplished 90, 100, 110, 120 and 130% of L-Arg requirements for each phases in Arg-deficient group and experimental groups, respectively.
Letters (a, b, c) in the same line indicate significant differences between different letters.
NS: Not significant

colon contractility are given in Table 3-6. Serum NO concentration is shown in Figure 1a,b,c.

It was observed that Arg in diet quadratic affected the serum IBD titer at grower phase. Accordingly, the highest serum IBD titer (P<0.05) was in the experimental group which was fed the diet containing 110% L-Arg, whereas there was no significant difference between other groups. Moreover, ND titer and H/L ratio did not differ at all three phases (Table 3).

The relative weight of spleen linearly increased in the experimental groups which were fed the diet containing 120 and 130% L-Arg at starter phase as compared to Arg-deficient group (P<0.05). It was detected that relative weight of bursa Fabricii increased at grower (P<0.05) and finisher (P<0.01) phases in the experimental group which was fed the diet containing 110% L-Arg as compared to 90 and 100% L-Arg and as compared to 90, 100 and 130% L-Arg, respectively. The relative weight of liver did not show any significant difference between groups (Table 4).

Arginine in diet showed quadratic effect on serum NO level. The lowest serum NO concentration (P<0.05) was seen in the Arg-deficient group at each three phases, while there was no significant difference between other groups (Figs. 1a,b,c).

The amplitude of mid colon motility did not show any significant differences between groups at starter, grower and finisher phases (Table 5). However, the frequency of contractions increased in Arg-deficient group at starter (P<0.05) and grower (P<0.01) phases. The highest frequency at finisher phase was observed in the groups which were fed the diet containing 90 and 100% L-Arg (P<0.05; Table 6).

Discussion

In this study, the effect of different levels of L-Arg on the antibody titers of IBD and ND, H/L ratio, organ development, serum NO concentration and the amplitude and frequency of mid colon contractions of broilers were investigated during the starter, grower and finisher phases. Arginine requirement of broilers was accomplished with the rate of 90-130% for three phases.

It has been reported that arginine improves the immune defence mechanism of the body against tumours and infectious agents (Lee et al. 2002). Moreover, Arg modulates or boosts humoral and cellular immune response to experimental infection challenges (Abdulkalykova and Ruiz-Feria 2006). In the present study, antibody titers against IBD and ND were not significant during the starter and finisher phases (Table 3). This finding was consistent with Kidd et al. (2001) who indicated that supplementation of 20% L-Arg above the level described in NRC to

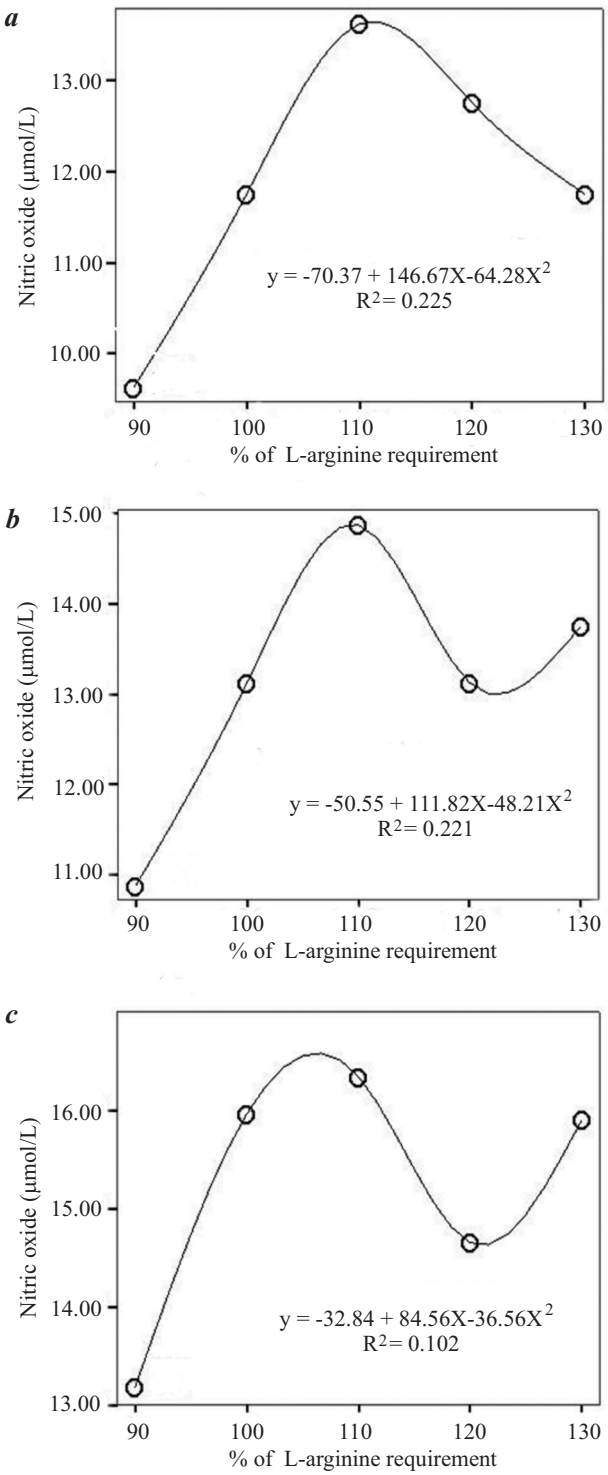

Fig. 1. Effect of different L-Arg levels on concentrations of nitric oxide during the starter (1A), grower (1B) and finisher (1C) phases (Mean ± SE)
These values accomplished 90, 100, 110, 120 and 130% of L-Arg requirements for each phases in Arg-deficient group and experimental groups, respectively.

the diet of vaccinated broiler chicks had no effect on plasma antibody concentrations. However, it was observed that the level of L-arg in diet showed a quadratic effect on serum IBD titer at grower phase ($P<0.05$; Table 3). This finding suggests that dietary L-Arg supplementation does not affect antibody synthesis after vaccination, especially at starter and finisher phases.

It has been stated that arginine supplements increase the proliferation of lymphocytes in the blood, boost suppressor T-cell counts (Barbul et al. 1981), phagocytic activity of alveolar macrophages (Tachibana et al. 1985). In this study, similarly to antibody titer, no significant difference in H/L ratio was observed (Table 3). The same finding was detected in other studies in which Arg was used at the rate of 90-120% (Kidd et al. 2001), 100-150% (Srinongkote et al. 2004) and 25-100% (Cengiz and Kucukersan 2010). However, it has been indicated that the diet supplemented with 175-200% L-Arg more than optimum Arg requirement increases H/L ratio in infected broilers during infectious bronchitis (Lee et al. 2002). The discrepancies between the present study and above mentioned reports suggest that the percentage of L-Arg in diet may affect H/L ratio during infection and does not alter this ratio in healthy animals.

The size and development of lymphatic organs are directly correlated with the health status of animals (Abdulkalykova and Ruiz-Feria 2006). Feeding the chickens with the diet which contains insufficient L-Arg level decreases the relative weight of bursa Fabricii (Kwak et al. 1999, Konashi et al. 2000). However, the diet supplemented with more than optimum Arg level increases the relative weight of spleen rather than bursa of Fabricius (Kwak et al. 1999). Besides, in other studies, it has been indicated that the levels of L-Arg in the diet does not affect the relative weights of spleen and bursa of Fabricius in broilers (Kidd et al. 2001, Abdulkalykova and Ruiz-Feria 2006, Cengiz and Kucukersan 2010). In the present study, it was observed that the relative weight of bursa Fabricii decreased in Arg-deficient group at grower and finisher phases in accordance with Kwak et al. (1999) and Konashi et al. (2000). Furthermore, the relative weight of spleen increased when L-Arg was supplemented to the diet at starter phase in accordance with Kwak et al (1999). Importantly, the present study provided evidence that the L-Arg content of diet at finisher phase had quadratic effect on the relative weight of bursa Fabricii and feeding with 90, 100 or 130% L-Arg had a negative effect on the relative weight of bursa fabricius ($P<0.01$; Table 4).

It has been reported that the immune stimulative effect of L-Arg supplementation may be mediated by increased NO formation. L-Arg is a precursor of NO and NO produced by L-Arg plays a role in defence mechanism against microorganisms. In the present study, the lowest NO level was observed in Arg deficient group in each three phases (Figs. 1a,b,c).

Moreover, a quadratic effect was determined between the L-Arg supplemented to diet and the level of blood NO concentrations. There are reports indicating that the supplementation with Arg levels exceeding those recommended do not alter blood NO concentrations in avian species (Zhao et al. 2009, Cengiz and Kucukersan 2010), as found in this study. This study provided evidence that lower L-Arg level had stronger effect on the level of blood NO rather than high level of L-Arg in diet.

The present study not only demonstrated the effect of Arg/NO pathway on immune system, but also focused on the GIS motility. It is well known that NO is a neuromediator of NANC nerve fibers. The relaxation of smooth muscle of intestine system is mediated by NO, which is released from NANC nerve fibers via NOS enzyme following electrical stimulation of intestine (Toda et al. 1990). Bulbul et al. (2013) reported that duodenum motility might be inhibited by NOS donor in broilers. In this study, the amplitude of mid colon contractions did not differ between groups (Table 5). However, it was observed that the frequency of colon contractions increased in Arg deficiency group at three phases (Table 6). The molecule orbit of NO contains unmapped electron-couple. Due to the short half-life free radical NO rapidly reacts with oxygen (Ignarro et al. 1987, Xie et al. 1992). Therefore, the similarity of spontaneous contractile tension of colon may be related to the absence of NO in these tissues due to short half-life of NO itself or the absence of L-Arg in Krebs's solution.

The present study provided evidence that the level of L-Arg in diet can be of importance for IBD antibody titer, lymphotic organ development, NO level and the frequency of colon contractility. In conclusion, it is suggested that increasing L-Arg level in diet supports the immune response due to producing NO at three phases in healthy broilers and this effect is more detectable when the diet contains 110% L-Arg at grower phase.

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