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RESEARCH ARTICLE

# The Effect of L-Arginine on Growth Performance, Some Serum Biochemical Parameters and Duodenal Motility in Broilers [1]

Tuba BULBUL [1] Zehra BOZKURT [2] Elmas ULUTAS [3] Oktay YILMAZ [4] Aziz BULBUL [3]

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[1] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, University of Afyon Kocatepe, TR-03200 Afyonkarahisar - TURKEY
[2] Department of Zootechnics, Faculty of Veterinary Medicine, University of Afyon Kocatepe, TR-03200 Afyonkarahisar - TURKEY
[3] Department of Physiology, Faculty of Veterinary Medicine, University of Afyon Kocatepe, TR-03200 Afyonkarahisar - TURKEY
[4] Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Afyon Kocatepe, TR-03200 Afyonkarahisar – TURKEY

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## Summary

The aim of this study was to evaluate the effect of diet supplemented with L-arginine (L-Arg)on growth performance, some serum biochemical parameters and duodenal motility of broilers during three time periods: 0 to 10, 11 to 28 and 29 to 42 days old. A total of 500, mixed sex, one-day-old Ross-308 broiler chicks were divided into five groups as follows: Arg deficient group and four experimental groups. Each group was then divided into five subgroups of 20 chicks each. Arg deficient group for all time periods was fed by basal diet which contained 10% less L-Arg than optimum Arg requirement recommended by the breeder. Experimental groups were fed by basal diet supplemented with L-Arg which was progressively 10% increased in groups. The highest body weight gain (BWG) was observed on days 11-28 and 0-42 in experimental group fed by basal diet supplemented with 110% L-Arg, whereas the lowest feed conversion ratio (FCR) was determined on days 29-42 and 0-42 in the same experimental group. Feed intake did not change in all three periods, while serum urea nitrogen level in the experimental group in which diet supplemented with 10% L-Arg, was lower than other groups on day 0-10. On contractility studies, it was observed that L-Arg inhibited the amplitude of contractions in duodenum in a dose-dependent manner *in vitro*. These results suggest that the basal diet formulated with 90-130% Arg is not effective on growth performance of chicks on days 0-10, whereas the diet supplemented with 10% L-Arg more than optimum Arg requirement is adequate during the days 11-28 and 29-42. Moreover, although the L-Arg decreased the duodenal contractility *in vitro,* it is suggested that the diet supplemented with 10% L-Arg more than optimum Arg requirement may be negatively affected the FCR in broilers.

***Keywords:*** *Broiler, L-arginine, Growth performance, Duodenal contractility*

# L-Arjininin Broylerlerde Büyüme Performansı, Bazı Biyokimyasal Parametreler ve Duodenal Motilite Üzerine Etkisi

## Özet

Bu araştırma 0-10., 11-28. ve 29-42. günler arasında broylerlerde L-arjinin (L-Arj)nin rasyonlara katılmasının büyüme performansı, bazı biyokimyasal parametreler ve duodenal motilite üzerine etkisini belirlemek amacıyla yapılmıştır. Araştırmada toplam 500 adet, bir günlük yaşta, karışık cinsiyette Ross 308 broyler civciv: Arj yetersiz grup ve dört deneme grubu olmak üzere beş gruba ayrılmıştır. Her bir grup da 20'şer civcivden oluşan 5 alt gruba ayrılmıştır. Arjininçe yetersiz grup tüm deneme boyunca üretici tarafından önerilen optimum Arj gereksiniminin %10'undan düşük L-Arj içeren temel rasyon ile beslenmiştir. Deneme grupları ise, temel rasyona %10 progresif artan düzeylerde L-Arj ilave edilen rasyonlarla beslenmiştir. Araştırma sonunda ihtiyacın %110 düzeyinde Arj bulunan diyetle beslenen grupta 11-28 ve 0-42. ($P<0.01$) günlerde en yüksek canlı ağırlık artışı (CAA), buna karşın aynı deneme grubunda 29-42. ve 0-42. günlerde en düşük yemden yararlanma oranı (YYO) bulunmuştur. Her üç dönemde YT gruplarda değişmezken serum üre azot düzeyi optimum Arj gereksiniminin %10 Arj ilaveli rasyondan oluşan deneme grubunda 10. günde diğer gruplara göre düşmüştür. *İn vitro* ortamda ise L-arjininin doza bağlı olarak duodenumda kasılımları baskıladığı görülmüştür. Sonuç olarak, broylerlerde arjinin gereksiniminin %90-130'unu karşılayacak şekilde hazırlanan rasyonların 0-10. günler arasında büyüme performansı üzerinde etkili olmadığı, ancak 11-28. ve 29-42. günler arası dönemlerde optimum Arj gereksiniminden %10 daha fazla L-Arj içeren rasyonun yeterli olduğu belirlenmiştir. Bununla birlikte, L-arjininin *in vitro* duodenum kasılımları azalttığı, bu bağlamda optimum L-Arj gereksinimin %10'nun daha fazla L-arjininli rasyonla beslenen broylerlerde YYO'nun olumsuz etkileneceği öngörülmektedir.

***Anahtar sözcükler:*** *Broyler, L-arjinin, Büyüme performansı, Duodenal kasılım*

**İletişim (Correspondence)**
+90 272 2281312
tbulbul@aku.edu.tr

# INTRODUCTION

It is important to add a balanced amount of Arg to poultry diets for growth development [1-3], nitrogen balance [3] and protein metabolism [4]. However, a decrease in body weight (BW) and BW gain (BWG), as well as in feed intake (FI) and feed conversion ratio (FCR), due to supplementation of Arg more than optimum Arg requirement of diet of broilers, has been reported [5]. Nitric oxide (NO) is synthesized from L-Arg in a two-step enzyme reaction by nitric oxide synthase (NOS) [6]. Nitric oxide is involved in the intestinal water transport by acting directly on the epithelium and blood flow or indirectly by stimulating neuronal reflexes. Noncholinergic nonadrenergic neural mechanisms involving nerves containing NO have been shown to modulate smooth muscle in the gastrointestinal tract and therefore NO may be important in the regulation of cyclical small intestinal motility [7]. Moreover, the nicotinamide adenine dinucleotide phosphate diaphorase activity in nerve fibres of jejunum in chickens has been reported [8]. It has been also well documented that the presence of neuronal NOS enzyme in nerve fibres of proventriculus in chickens [9].

Previous studies in broiler chickens have demonstrated the effect of adequate or higher Arg levels on growth performance [5,10,11]. However, to the authors' knowledge, there have been no reports 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 intestinal motility and NO metabolism. Therefore, the present study was designed to evaluate the effect of different levels of Arg on growth performance, serum biochemical parameters and duodenal motility in 0-10, 11-28 and 29-42 days old broilers.

# MATERIAL and METHODS

### *Animals, Housing and Experimental Desing*

This study was carried out at the Animal Research Center of Afyon Kocatepe University, after approval by the local ethical committee (B.30.2.AKU.09.Z.010). Five hundred, one-day old, mixed sex chicks (Ross-308) were obtained from a commercial hatchery, weighed and randomly separated into five 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 h of light and 1 hour of dark throughout the study.

The chicks were reared on the floor of pens in a curtain-sided broiler house. Pine wood shavings were used as litter material. The pens were 2 $m^2$ in size and the stocking density was 12 chicks per square meter. 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 from 0 to 10, 11 to 28 and 29 to 42 day old. The diet of Arg deficient group for all time periods was consisted of basal diet which was formulated 10% less L-Arg than optimum Arg requirement recommended by the breeder. The Arg-deficient diet contained 1.35, 1.14 and 0.99% Arg for all time periods, respectively. All nutrients met or exceeded the nutrient requirements for broiler chickens [12]. The levels of crude protein (CP) and Arg of the basal diet, including corn, corn gluten, soybean meal, full-fat soybean and meat and bone meal, were analysed before being used in formulation *(Table 1)*. 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 levels of CP and amino acids of the feed ingredients, including corn, corn gluten, soybean meal, full-fat soybean and meat and bone meal, were calculated before being used in the design of the diet formulations. The CP levels in the diets and raw feed materials was analysed using methods of AOAC [13]. L-Arg levels in the diet were determined by LC-MS-MS (Applied Biosystems API-3200) in a laboratory (ANT Technical Devices Lab.) *(Table 1)*.

### *Growth Performance*

The mortality rate of broilers was recorded daily. Body weights were recorded by pen on days 0, 10, 28, and 42. Feed intake and BWG per pen was recorded 0-10, 11-28 and 29-42 days. Feed conversion rations were calculated by dividing the cumulative feed intake per pen by the live body mass per pen at the end of the measurement periods.

### *Biochemical Parameters*

Blood samples were collected into non-heparinized tubes from 10 birds from each group (2 birds/replicate) on days 10, 28 and 42 during sacrifying and serum was collected by centrifugation. Serum was harvested and stored (−20°C) before analysis. Sera were analysed for concentrations of alkaline phosphatase (ALP), alanine aminotranspherase (ALT), aspartate aminotranspherase (AST), creatinine, urea nitrogen (BUN)in an autoanalyzer (Tokyo Boeki Prestige 24i, Japan).

### *The Preparation of Isolated Smooth Muscle Strips of Duodenum for Contractility Experiments*

Duodenum was 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 were continuously ventilated with a gas mixture (95% $O_2$ and 5% $CO_2$). Five mm-long ring strips of samples were

***Table 1. C****omposition of the Arg deficient diets in different periods (%)*
***Tablo 1.*** *Farklı dönemlerde Arj yetersizliği oluşturulan diyetlerin bileşimi (%)*

| Ingredients | Phase (days) | | |
|---|---|---|---|
| | 0 to 10 d | 11 to 28 d | 29 to 42 d |
| Corn | 53.53 | 55.38 | 57.22 |
| Corn gluten meal | 6.60 | 9.00 | 9.00 |
| Boncalite | - | - | 5.00 |
| Soybean meal | 14.75 | 3.20 | 0.43 |
| Full fat soybean | 17.14 | 24.00 | 19.63 |
| Meat bone meal | 4.00 | 4.00 | 4.50 |
| Vegetable oil | 1.50 | 2.00 | 2.50 |
| Calcium carbonate | 0.64 | 0.60 | 0.28 |
| Dicalcium phosphate | 0.62 | 0.55 | 0.25 |
| Salt | 0.19 | 0.20 | 0.20 |
| DL-Methionine | 0.22 | 0.20 | 0.07 |
| L-Lysine HCl | 0.31 | 0.37 | 0.27 |
| Sodium bicarbonate | 0.20 | 0.20 | 0.20 |
| Vitamin premix* | 0.20 | 0.20 | 0.30 |
| Mineral premix** | 0.10 | 0.10 | 0.15 |
| Calculated composition, % | | | |
| ME, kcal/kg | 3023 | 3159 | 3173 |
| Crude protein | 22.20 | 20.80 | 19.00 |
| Calcium | 0.97 | 0.92 | 0.80 |
| Available phosphorus | 0.46 | 0.44 | 0.42 |
| Methionine + Cystine | 0.99 | 0.96 | 0.80 |
| Lysine | 1.31 | 1.24 | 1.04 |
| Arginine | 1.38 | 1.21 | 1.01 |
| Analyzed composition, % | | | |
| Crude protein | 22.14 | 21.57 | 19.65 |
| Arginine | 1.35 | 1.14 | 0.99 |

** Vitamin premix provides per 2.5 kilogram of diets: 12.000.000 IU vitamin A, 2.500.000 IU vitamin $D_3$, 40.000 mg vitamin E, 5.000 mg vitamin $K_3$, 3.000 mg vitamin $B_1$, 6.000 mg vitamin $B_2$, 5.000 mg vitamin $B_6$, 20 mg vitamin $B_{12}$, 25.000 mg niacin, 12.000 mg pentatonic acid, 1.000 mg folic acid, 50 mg biotin, 10.000 mg BHT, ** Mineral premix provides 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*

dissected from the middle point 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 KS (pH 7.4), which were continuously oxygenated (95% $O_2$ and 5% $CO_2$) at 37°C. The isometric smooth muscle activity of duodenum 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 Duodenum Contractility***

Duodenum 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 1 g for the strips. After the completion of the 30 min baseline period, contractions of longitudinal strips of duodenum for each animal were visualized and recorded to determine normal spontaneous contractions. Thereafter, the strips were treated with Arg (Sigma, Cat # A8094) at increasing concentration ($10^{-5}$ – $10^{-3}$ M) to determine endogenous NO

activity. The mean tension of spontaneous contractions for each strip calculated for a 10-min period before administration of examined substances was set as 100% (control period). Thereafter, changes in contractions caused by the examined substances were recorded and compared to the control period [14,15].

***Statistical Analysis:*** Data from treatment means were analyzed as a completely randomized design using the General Linear Models procedure of the SPSS for windows. When differences (P<0.05) among 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.

## RESULTS

Broilers fed graded levels of Arg had showed quadratic responses for BWG in 11 to 28 and 0 to 42 d of age, FCR in the 29 to 42 and 0 to 42 d of age.

It was observed that BWG on days 11-28 (P<0.05) and 0-42 (P<0.01) in experimental group which chicks were fed with diet contained 110% Arg increased when compared to experimental diet groups contained 90 and 130% Arg. Similarly, FCR was more adequate in the same groups on days 29-42 and 0-42 (P<0.001). Feed intake did not show any significant difference between groups in all time periods *(Table 2)*.

It was determined that the levels of serum ALP, ALT, AST and CRE did not differ between groups, while BUN in the experimental group in which diet supplemented with 10% L-Arg, was lower than other groups on day 0-10 *(Table 3)*.

The amplitude of spontaneous contractility of duodenum in groups did not show any significant difference during the days 0-10, 11-28 and 29-42 *(Table 4)*. However, increasing concentrations of Arg from $10^{-5}$ to $10^{-3}$ M decreased the amplitude of duodenal contractility in all time periods (P<0.001). Moreover, the most effective level of Arg in decreasing the amplitude of duodenal contractility was $10^{-3}$ M *(Fig. 1A, B, C)*. The percentage of inhibition of contractility analysis showed no significant differences in all groups in the days 0-10, 11-28 and 29-42 *(Table 4)*.

## DISCUSSION

The present study was performed in three time periods. Arg requirement of broilers was provided by the diet which was 10% less than optimum Arg level recommended by the breeder and 10% progressive increments of Arg for each experimental group. Consequently, Arg requirement was accomplished with the rate of 90-130% on days 0-10, 11-28 and 29-42 in broilers. It has been reported that Arg in diet is required for optimum BWG [2,4,16,17], however the level of Arg below requirement [16] or 25% higher than requirement in diet [5,11] decreases BWG. Burton and Waldrop [18], Cuca and Jensen [1] and NRC [19] (1994) have suggested that the sufficient Arg level in diet is 1.25-1.40% until the day 28, 1.24 to 1.28% in first three weeks and 1.25% between days 0-21, 1.1% between days 22-42, respectively. In the present study, we observed that BWG increased in experimental group which the Arg requirement corresponded to 110% Arg on days 11-28 and 0-42 when compared to other experimental groups (P<0.01).

In the current study, FI did not differ in groups in each time periods. This result was consistent with Kidd et al.[2] and Corzo et al.[3] who suggested that various levels of L-Arg

***Table 2.*** *The growth performance of broilers fed by the diet which consisted of 90, 100, 110, 120 and 130% of Arg requirement*
***Tablo 2.*** *Arj ihtiyacının %90, 100, 110, 120 ve 130'unun karşılandığı diyet ile beslenen broylerlerin büyüme performansı*

| Growth Performance | Days | Treatment | | | | | SEM | P | |
|---|---|---|---|---|---|---|---|---|---|
| | | 90 | 100 | 110 | 120 | 130 | | Linear | Quadretic |
| Body weight gain, g | 0 to 10 | 225.3 | 227.6 | 230.7 | 225.4 | 220.2 | 1.56 | 0.263 | 0.316 |
| | 11 to 28 | 983.3b | 1029ab | 1060a | 1015ab | 998.1b | 8.49 | 0.763 | 0.014* |
| | 29 to 42 | 965.0 | 1004 | 1032 | 1008 | 949.8 | 11.49 | 0.723 | 0.078 |
| | 0 to 42 | 2173b | 2261ab | 2322a | 2248ab | 2168b | 17.03 | 0.803 | 0.004** |
| Feed intake, g | 0 to 10 | 297.7 | 294.8 | 291.5 | 296.7 | 300.3 | 3.17 | 0.829 | 0.856 |
| | 11 to 28 | 1662 | 1781 | 1731 | 1747 | 1739 | 15.14 | 0.250 | 0.140 |
| | 29 to 42 | 2938 | 2708 | 2646 | 2697 | 2745 | 30.86 | 0.107 | 0.133 |
| | 0 to 42 | 4896 | 4785 | 4669 | 4741 | 4785 | 38.76 | 0.329 | 0.453 |
| Feed conversion ratio, g/g | 0 to 10 | 1.32 | 1.29 | 1.26 | 1.31 | 1.36 | 0.012 | 0.237 | 0.102 |
| | 11 to 28 | 1.69 | 1.73 | 1.63 | 1.72 | 1.74 | 0.013 | 0.254 | 0.062 |
| | 29 to 42 | 3.05a | 2.69c | 2.56c | 2.67c | 2.89b | 0.04 | 0.061 | 0.000*** |
| | 0 to 42 | 2.25a | 2.11bc | 2.01c | 2.10bc | 2.20ab | 0.02 | 0.340 | 0.000*** |

*Letters (a, b, c) indicate significant differences between them in each column, * P<0.05, ** P<0.01, *** P<0.001*

***Table 3.*** *Effects of Arg on levels of serum alkaline phosphatase (ALP), alanin aminotranspherase (ALT), aspartat aminotranspherase (AST), creatinin, urea nitrogen (BUN) on days 10th, 28th and 42nd*

***Tablo 3****. Serum alkalen fosfataz (ALP), alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), kreatinin ve üre azot (BUN) düzeyi üzerine 10., 28. ve 42. günlerde Arj'in etkisi*

| Parameters | Day | Treatment | | | | | SEM | P | |
|---|---|---|---|---|---|---|---|---|---|
| | | 90 | 100 | 110 | 120 | 130 | | Linear | Quadretic |
| ALP, U/L | 10 | 21.21 | 18.50 | 10.51 | 11.12 | 13.21 | 6.33 | 0.458 | 0.433 |
| | 28 | 631.3 | 765.5 | 443.0 | 634.2 | 773.6 | 121.3 | 0.919 | 0.128 |
| | 42 | 976.4 | 1253 | 1388 | 1360 | 1335 | 87.33 | 0.574 | 0.314 |
| ALT, U/L | 10 | 4.87 | 2.75 | 2.50 | 3.50 | 3.50 | 0.87 | 0.157 | 0.98 |
| | 28 | 3.00 | 1.75 | 2.50 | 2.37 | 2.14 | 0.12 | 0.357 | 0.210 |
| | 42 | 2.80 | 3.00 | 2.28 | 2.12 | 2.71 | 0.22 | 0.709 | 0.460 |
| AST, U/L | 10 | 159.8 | 182.0 | 154.1 | 170.4 | 148.5 | 15.43 | 0.293 | 0.356 |
| | 28 | 194.3 | 152.2 | 168.0 | 151.5 | 153.3 | 7.22 | 0.062 | 0.376 |
| | 42 | 210.6 | 249.3 | 238.9 | 283.2 | 308.8 | 12.74 | 0.128 | 0.783 |
| Creatinin, mg/dl | 10 | 0.10 | 0.12 | 0.07 | 0.11 | 0.12 | 0.01 | 0.742 | 0.567 |
| | 28 | 0.061 | 0.03 | 0.07 | 0.03 | 0.04 | 0.01 | 0.156 | 0.098 |
| | 42 | 0.14 | 0.12 | 0.12 | 0.12 | 0.13 | 0.04 | 0.316 | 0.060 |
| BUN, mg/dl | 10 | 5.73a | 6.37a | 4.16b | 6.60a | 5.71a | 0.28 | 0.019* | 0.021* |
| | 28 | 3.50 | 2.50 | 3.71 | 3.12 | 2.71 | 0.44 | 0.238 | 0.567 |
| | 42 | 4.50 | 3.75 | 4.42 | 4.00 | 3.71 | 0.33 | 0.068 | 0.256 |

*Letters (a, b) indicate significant differences between them in each column, * P<0.05*

***Table 4.*** *The amplitude of spontaneous contractility of duodenum on days 10th, 28th and 42nd*

***Tablo 4.*** *Spontan duodenum kasılımlarının 10., 28. ve 42. günlerde amplitüdü (g)*

| The amplitude of Spontaneous Contractility (g) | | Treatment | | | | | SEM | P |
|---|---|---|---|---|---|---|---|---|
| | | 90 | 100 | 110 | 120 | 130 | | |
| Day | 10 | 0.50 | 0.52 | 0.48 | 0.53 | 0.50 | 0.022 | 0.975 |
| | 28 | 0.62 | 0.68 | 0.69 | 0.61 | 0.56 | 0.028 | 0.589 |
| | 42 | 0.70 | 0.76 | 0.71 | 0.63 | 0.64 | 0.026 | 0.685 |

supplementation did not show any effect on FI in broilers. In contrast to our finding, it has been reported that Arg-deficient diet [16] or high level of Arg in diet [3,5] decreased the FI in broilers. However, Skalan and Plavnik [20] reported that the effect of Arg on FI and BWG was related to protein level in diet. Therefore, it is suggested that discrepancy in FI and BWG in above-mentioned reports may be different CP levels in diet.

We clearly demonstrated that the level of Arg corresponded to 90-130% of Arg requirement did not alter FCR on days 11-28 and 0-10 however, FCR was more adequate in the experimental group which the diet contained 110% Arg on days 29-42 and 0-42. This finding supports the previous observations that either Arg deficiency [2,16] or high level of Arg in diet [5,21] decreases the FCR level. Moreover, it has been emphasised that the Arg:lysine ratio in diet should be balanced between days 21 and 42 and FCR may be negatively affected by the changes of Arg:lysine ratio [22]. In the present study, the discrepancies of FCR in Arg deficient group and the diet group which contained 130% Arg during the 29 to 42 day period may be explained by the occurrence of alteration in the Arg:Lysine ratio in diet.

In the present study, serum ALP, ALT and AST levels did not show any significant difference between groups. Besides the serum concentrations of ALP, ALT and AST are valuable indicators to detect any abnormality of liver [22], it is suggested that the presence of Arg in diet corresponded to 90-130% of Arg requirement is ineffective on liver enzymes.

Creatinine is mainly released from liver and pancreas and excreted from the body via glomerular filtration and tubular secretion. Therefore, creatinine is a gold standard for kidney damage, when compared to BUN [23]. There was statistically significant difference in BUN concentration in the current study (if it is not 2 fold higher than normal value) [22]. Arginine did not produce important difference in terms of kidney function test *(Table 3)*. In the current study, a significant difference was observed in the concentrations of BUN levels between groups on day 0-10,

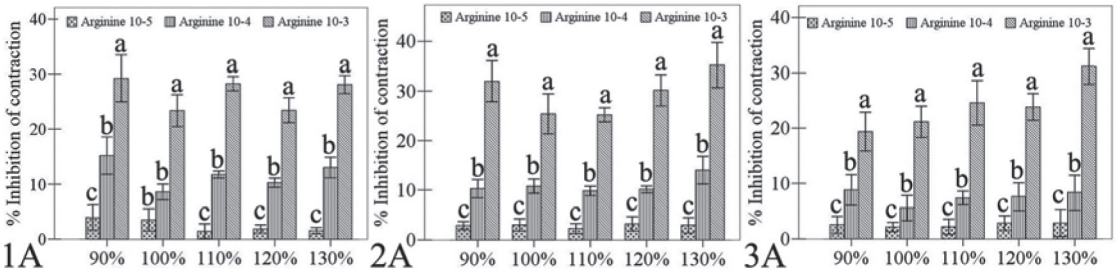


**Fig 1.** Percentage inhibition of spontaneous contractions in duodenum on 10th, 28th and 42nd days by application of Arg at doses of $10^{-5}$, $10^{-4}$ and $10^{-3}$ M

Letters (a,b,c) indicate significant differences between them in each column. *P*-value was <0.001 in groups determined by ANOVA. 1A) 0 to 10 d of age, 1B) 11 to 28 d of age 1C) 29 to 42 d of age

**Şekil 1.** Duodenumda 10., 28. ve 42. günlerde Arj'in $10^{-5}$, $10^{-4}$ and $10^{-3}$ M dozlarının spontan kasılımlarda oluşturduğu yüzde inhibisyon

Her bir kolonda farklı harfler (a,b,c) istatistiksel önemi gösterir. *P*-değeri ANOVA tarafından gruplarda < 0.001 belirlendi. 1A) 0-10 günlük yaş, 1B) 11-28 günlük yaş,1C) 29-42 günlük yaş

***Table 5.*** *Percentage inhibition spontaneous contractions in groups in the presence of Arg ($10^{-5}$, $10^{-4}$, $10^{-3}$ M) on isolated strips of duodenum on day 10th, 28st and 42nd*

***Tablo 5.*** *Gruplarda 10., 28. ve 42. günlerde izole duodenum örneklerinde Arj ($10^{-5}$, $10^{-4}$, $10^{-3}$ M) spontan kasılımlarda oluşturduğu yüzde inhibisyon*

| Inhibition Spontaneous Contractions (%) | Treatment | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | 90 | 100 | 110 | 120 | 130 | | |
| **10th day** | | | | | | | |
| Arg$10^{-5}$ M | 2.40 | 3.49 | 2.02 | 1.84 | 1.55 | 0.48 | 0.784 |
| Arg$10^{-4}$ M | 11.30 | 8.55 | 11.57 | 10.24 | 11.24 | 0.62 | 0.570 |
| Arg$10^{-3}$ M | 30.86 | 23.38 | 28.28 | 23.44 | 28.12 | 1.13 | 0.140 |
| **28th day** | | | | | | | |
| Arg$10^{-5}$ M | 2.87 | 3.00 | 2.28 | 3.21 | 2.94 | 0,49 | 0.987 |
| Arg$10^{-4}$ M | 2.86 | 10.28 | 10.78 | 9.83 | 10.12 | 0.76 | 0.440 |
| Arg$10^{-3}$ M | 31.97 | 25.41 | 25.20 | 30.17 | 35.20 | 1.69 | 0.280 |
| **42nd day** | | | | | | | |
| Arg$10^{-5}$ M | 1.90 | 2.14 | 2.17 | 2.86 | 2.86 | 0.61 | 0.967 |
| Arg$10^{-4}$ M | 7.17 | 5.56 | 7.33 | 7.60 | 8.32 | 0.98 | 0.946 |
| Arg$10^{-3}$ M | 21.56 | 21.14 | 24.59 | 23.83 | 31.17 | 1.51 | 0.220 |

whereas creatinine did not show any significant difference between groups. It has reported that if these enzymes does not increase two fold higher as compared to their normal values, no kidney damage would be expected [23]. Therefore, it is suggested that no changes in kidney function occurs due to Arg in diet.

Nitric oxide was reported to be an important regulator of gastrointestinal motility [24-26]. It has been reported that NO is an inhibitor neurotransmitter in canine isolated ileocolonic junction and longitudinal smooth muscle of duodenum [25] and rat gastric fundus [26]. It has been also reported that oral administered Arg have a regulatory role in esophageal and gall bladder motility [27,28]. Moreover, it has been demonstrated that intravenous or intragastric administration of large amounts of Arg delays gastric emptying in humans [29,30] and dogs [31]. Therefore, the present study also focused on the effect of Arg supplemented to diet on motility of duodenum of chickens throughout three time periods. In the present study, spontaneous contractions of duodenum did not change on days 0-10, 11-28 and 29-42 *(Table 4)*. It is suggested that this may be attributed to the metabolisation of Arg before reaching the duodenum or to the absence of NO in these tissues due to short half-life of NO itself.

Arginine shows its relaxing effect on smooth muscles via self-conversion to NO by using NO synthase in these tissues [27]. It has been observed that L-Arg completely inhibits smooth muscle contractility at doses of 0.01–1 mM, *in vitro* [32]. Bulbul et al.[14] demonstrated that the most effective dose of L-Arg was $10^{-5}$ M in rat intestinum. In the present study, Arg inhibited the spontaneous contractions of duodenum and this finding is consistent with above-mentioned reports. However, it was observed the inhibition rate did not show any significant difference between

groups. The tissue or serum NO concentrations are changed by the alteration of NO synthase expression [33]. Similarly, it has already been reported that SNP, a exogenous NO donor and L-NAME, a selective NOS inhibitor changes the nNOS expression in jejunum but not in duodenum and ileum in broilers [34]. In this study, no difference was observed in the inhibition of duodenum contractility between groups, *in vitro*. Therefore, it is suggested that oral administration of Arg may not be affected by intestinal NOS enzymes.

In conclusion, in agreement with the contractility data that dietary Arg affected the motility in the duodenum by decreasing the amplitude of contraction, it is indicated that the diet supplemented with 110% Arg is found to be satisfactory between days 11-42 in broiler nutrition.

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