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Research Article

Effect of Guanidine Acetic Acid Addition to Corn-Soybean Meal Based Diets on Productive Performance, Blood Biochemical Parameters and Reproductive Hormones of Laying Hens

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Abstract

The present study was conducted to investigate the effects of GAA addition to corn-soybean meal based diets on productive performance, blood biochemical parameters and reproductive hormones of laying hens. Two hundred laying hens were used in a completely randomized design with 5 treatments and 4 replicates (n=10 birds). Birds received basal diets containing 0 (negative control), 0.057, 0.114 and 0.171% GAA/kg of diet. A diet containing 2% meat meal was also considered as positive control. The data for productive performance was recorded during 84 d and blood samples were collected to evaluate the blood biochemical parameters including triglycerides, high and low density lipoproteins, aspartate aminotransferase and alanine aminotransferase and nitric oxide and reproductive hormones (LH and FSH) at 42 and 84 d. Results showed that GAA addition to diet had not significant effects on productive performance but 0.171% GAA addition reduced egg weight ($P<0.05$) compared with control groups. Also, 0.114% GAA addition to diet increased levels of LH and FSH compared with control groups at 42 and 84 days. It can be concluded that GAA addition to diet is not appropriate strategy for improving performance of laying hens.

Keywords: *Creatine, Guanidine acetic acid, Laying hens, Nitric Oxide, Productive performance, Reproductive hormones*

Mısır-Soya Küspesi Diyetine Eklenen Guanidino Asetik Asitin Yumurta Tavuklarının Üretim Performansı, Kan Biyokimyasal Parametreleri ve Üreme Hormonlarına Etkisi

Özet

Bu çalışma GAA'nın mısır soya fasulyesi ağırlıklı rasyonlara eklenmesinin üretim performansı, kan biyokimyasal parametreleri ve yumurta tavuklarının üreme hormonları üzerindeki etkilerini araştırmak amacıyla yürütülmüştür. Çalışma tamamen rastgele edilmiş bir tasarım ile 5 grup ve 4 alt grup (n = 10 tavuk) iki yüz yumurta tavuğu ile yürütülmüştür. Tavuklar 0 (negatif kontrol), 0.057, 0.114 ve %0.171 GAA/kg içeren bazal rasyon ile beslenmiştir. Ayrıca rasyonda %2 et unu içeren bir de pozitif kontrol grubu oluşturulmuştur. Üretim performans verileri 84 gün boyunca kaydedildi ve trigliseridler, yüksek ve düşük yoğunluklu lipoproteinler, aspartat aminotransferaz, alanin aminotransferaz, nitrik oksit ve üreme hormonları (LH ve FSH) başta olmak üzere kan biyokimyasal parametrelerini değerlendirmek için 42 ve 84. günlerde kan numuneleri alındı. Sonuç olarak GAA'nın tavuk rasyonuna ilave edilmesiyle, üretim performansı üzerinde anlamlı bir etkisinin olmadığı, %0.171 GAA katkısının kontrol grubuna kıyasla yumurta ağırlığını azalttığı ($P<0.05$) görüldü. Ayrıca, rasyonda %0.114 GAA ilavesi ile kontrol gruplarına kıyasla LH ve FSH düzeylerinde artış görülmüştür. GAA'nın rasyona eklenmesinin yumurta tavuklarında performansını arttırmak için uygun bir strateji olmadığı sonucuna varıldı.

Anahtar sözcükler: *Kreatin, Guanidin asetik asit, Yumurta tavuğu, Nitrik Oksit, Üretim performansı, Üreme hormonları*

INTRODUCTION

Guanidine acetic acid (GAA) is synthesized from arginine and glycine and it is precursor for creatine synthesis [1]. Studies have shown that by 50% of daily creatine requirements is supplied through *de-novo* synthesis and the rest part by the feed [2]. It has been known lack of creatine in corn-soybean meal based diets [3,4]. It seems

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that lack of creatine reduces cell performance and finally productive performance in animals because of its role in metabolism.

Studies have shown that GAA addition to diets increases its conversion to creatine and this conversion was evidenced by increase in muscle and blood creatine [5-7]. GAA addition to diet is beneficial because it has much amounts arginine which may be spared [8,9]. Studies have shown that GAA spares arginine in deficient-arginine diets and it additionally improves growth performance in enough-arginine diets [7]. Earlier studies have shown the importance of arginine for nitric oxide and protein synthesis, growth and development in vertebrata [10,11]. Nitric oxide releases gonadotropin-releasing hormone (GnRH) by activating pituitary nitric oxide synthase which finally influences FSH and LH hormones [12]. Studies have indicated that FSH is involved in the recruitment of new follicles into the ovarian hierarchy and also the maturation and rapid growth of these new follicles [13,14]. A study has shown that FSH and LH hormones increase oviduct secretions and egg weight and also improve feed conversion ratio in laying hens [15]. It was reported a positive correlation between LH and ovulation [16]. Considering sparing effect of arginine by GAA, Basiouni [17] stated that arginine increases LH hormone secretion and subsequently improves productive performance in laying hens. On the other hand, arginine reduces abdominal lipid and balances lipid storage by nitric oxide synthesis [18]. Yang *et al.*[19] showed that dietary inclusion of arginine significantly reduced levels of liver aspartate amino-transferase in laying hens, implicating that arginine reduces liver damages. GAA is able to sparing body pool of arginine which is used for creatine synthesis [20] since it is converted to creatine in bird's kidney and liver [21]. Any study has been not still investigated effects of GAA on productive performance and reproductive hormones in laying hens. This study was conducted to investigate of several hypotheses including 1) GAA, as creatine precursor, acts and may improve energy requirements, if needs are faulted. 2) GAA is consisted of arginine and glycine and considering previous studies; does it spares and produces arginine in laying hens? 3) Considering relation between GAA and nitric oxide and as well as effects of nitric oxide on reproductive hormones and lipids, does it improves lipid and reproductive hormones by nitric oxide? Thus, the present study was conducted to investigate the effects of GAA addition to corn-soybean meal based diets (lack of creatine) on productive performance, blood biochemical parameters and reproductive hormones of laying hens.

MATERIAL and METHODS

Animal and Feed Material

All the used experimental protocols were approved by the guidelines of the Animal Ethics Committee of Science and Research University (Tehran, Iran). This experiment was conducted in house hen poultry farm placing in Shahryar town (Tehran-Iran). Two-hundred Hy-Line W-36 laying hens, 29 weeks of age and with mean body weight of 1410±100 g, were allocated to a completely randomized design with 5 treatments and 4 replicates (n=10 birds). One week adaption period was considered and birds were 30-old-week of age in start of experiment. The experimental period was lasted for 12 weeks. The rearing conditions were similar for all birds. Laying hens were kept in a layer house with 2 hens in each battery cage (50×50 cm) and each 5 cage were considered as a replicate. The birds were maintained under 16L:8D lighting program at all the experiment. Housing temperature and relative humidity were kept at 18°C and 45-50%, respectively. Water and feed were *ad libitum* supplied at all the study by drinkers and feeders, respectively. CreAmino®, produced by Evonic Industry Co, was used as GAA source which was containing 96% GAA, 1% water and 1% starch. Nutritional matrix of CreAmino® was as follows; 100% digestibility, 221% crude protein, 77% arginine and 83.000 kcal/kg AMEn. Birds received basal diets supplemented with different levels of GAA (0, 0.057, 0.114 and 0.171%). Two control diets, negative and positive, were also considered. Experimental treatments were as follows; 1) corn-soybean meal based diets without supplement as negative control, 2) corn-soybean meal based diets containing 0.057% GAA, 3) corn-soybean meal based diets containing 0.114% GAA, 4) corn-soybean meal based diets containing 0.171% GAA and 5) diets containing 2% meat meal as positive control. Experimental diets were prepared on the basis Hy-Line W-36 catalogue. The ingredients and composition of the basal diet are presented in *Table 1*. The proximate analyses of some nutrients were performed according to Association of official Analytical Chemists, or AOAC [22]. The amounts calculated of GAA were firstly mixed small amounts of the basal diet as a small batch, and then added to the calculated amount of the basal diet to obtaining a homogenous diet. The feed was prepared in mash form for all birds at same place.

Productive Performance

Egg production (EP), feed intake (FI), and egg weight (EW) was daily recorded during 1-84 days of trial, from each cage and egg mass (EM: EP×EW/100) feed conversion ratio (FCR: FI/EM) and were biweekly calculated. Body weight (BW) was measured at start and end of trial to measure the changes.

Blood Sampling and Measurement of Hormones

At 42 and 84 days of trial, blood samples (3 mL) were collected from 1 bird each replicate and centrifuged at 2500×g for 15 min. Levels of LH (CAT No. ZB-0014-Ch9648), FSH (CAT No. ZB-0012-Ch9648), nitric oxide (CAT No. ZB-NO-48A, V406), aspartate aminotransferase (CAT No. ZB-0143-Ch9648) and alanine aminotransferase (CAT No. ZB-0131-Ch9648) were measured using ZellBio® GmbH

Table 1. *Composition of experimental diets*					
Ingredient (%)	**Negative Control**	**0.057% GAA**	**0.114% GAA**	**0.171% GAA**	**Positive Control**
Corn	63.60	63.54	63.48	63.42	64.00
Soybean meal	23.00	23.00	23.00	23.00	21.00
GAA	0.00	0.057	0.114	0.171	0.00
Meat meal	0.00	0.00	0.00	0.00	2.00
Soybean oil	1.20	1.203	1.206	1.209	0.80
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.20
Limestone	9.60	9.60	9.60	9.60	9.60
Salt	0.20	0.20	0.20	0.20	0.20
Soda	0.25	0.25	0.25	0.25	0.25
Potassium carbonate	0.25	0.25	0.25	0.25	0.25
Premix[1]	0.50	0.50	0.50	0.50	0.50
Methionine	0.16	0.16	0.16	0.16	0.16
Lysine	0.02	0.02	0.02	0.02	0.02
Choline chloride	0.02	0.02	0.02	0.02	0.02
***Nutrient analysis* (calculated)**					
ME, kcal/kg	2571.39	2571.39	2571.39	2571.39	2571.39
Crude protein, %	15.93	15.93	15.93	15.93	15.93
Crude fiber, %	15.93	15.93	15.93	15.93	15.93
NFE, %	3.90	3.90	3.90	3.90	3.90
Anion-cation, kg/mEq	202.15	202.15	202.15	202.15	202.15
Ca, %	3.89	3.89	3.89	3.89	3.89
Available P, %	0.29	0.29	0.29	0.29	0.29
Sodium, %	0.16	0.16	0.16	0.16	0.16
Methionine,%	0.39	0.39	0.39	0.39	0.39
Lysine, %	0.74	0.74	0.74	0.74	0.74
Methionine+ cysteine,%	0.62	0.62	0.62	0.62	0.62
Threonine %	0.53	0.53	0.53	0.53	0.53

[1] Provided per kilogram of diet: Vit. A, 5000 IU; Cholecalciferol, 750 IU; Vit. E, 7.5 mg; Menadione, 0.63 mg; Thiamine, 0.25 mg; Riboflavin, 1.60 mg; Pyridoxine, 0.500 mg; Vit. B_{12}, 4.0 µg; Niacin, 12.5 mg; Calcium pantothenate, 1.8 mg; Butylated hydroxytoluene, 63 mg, Iron, 44 mg; Iodine, 1.2 mg; Cobalt, 0.36 mg; Selenium, 0.24 mg

commercial kits and by ELISA method as recommended by Producer Company. The serum concentrations of triglycerides, high and low density lipoprotein were measured by Pars Azmun commercial kits (Tehran-Iran).

Statistical Analysis

A completely randomized experimental design (CRD) were applied and the data were subjected to statistical analysis using analysis of variance (ANOVA) appropriate for a. When significant effects were detected by ANOVA, treatment means were compared using Duncan's multiple range test. All statistical analyses were performed with SAS [23]. Differences were considered significant at P<0.05. All of the parameters were analyzed as follows;

$$Yij = \mu + Ti + eij$$

Where Yij is the individual observation, µ is the overall mean, Ti is the effect of treatment, and eij represents the random error.

RESULTS

Tables 2, 3, 4, 5 and *6* show the effects of GAA addition to diet on productive performance. During experiment and in total**,** the added different levels of GAA to diet did not change FI, FCR, EP and EM in comparison with positive and negative controls (P>0.05); showing that dietary inclusion of meat meal and GAA have not significant effects on FI, FCR, EP and EM of laying hens. EW was reduced from 2th week to 12th week in laying hens fed the 0.171% GAA compared with other groups (P<0.05). Dietary inclusion of 0.114% GAA to diet significantly (P<0.05) increased levels of LH and FSH compared with negative and positive controls at 42 and 84 d *(Table 7)*. Blood biochemical parameters were not influenced by GAA addition supplement (P>0.05; *Table 8)*.

DISCUSSION

Findings showed that GAA addition to diet had not significant effects on productive performance but 0.171% GAA addition decreased egg weight from 2th week to

Table 2. *Effects of GAA addition to diet on FI (g) of laying hens at different periods*							
Groups	**Negative Control**	**Positive Control**	**0.057% GAA**	**0.114% GAA**	**0.171% GAA**	**P**	**SEM**
1-14d	87.75±4.65	86.49±5.61	86.25±6.01	86.31±5.73	81.75±3.53	0.844	0.321
15-28d	89.38±3.77	86.75±5.70	86.75±3.37	87.50±2.87	85.50±4.50	0.457	0.656
29-42d	86.38±4.30	85.38±6.20	81.50±5.42	86.63±3.24	82.38±4.37	0.129	0.737
43-56d	87.00±2.77	84.63±5.52	83.00±4.20	85.75±2.52	87.25±1.98	0.131	0.707
57-70d	91.21±2.02^{a}	89.09±3.12^{a}	84.50±4.50^{b}	88.00±3.42^{a}	88.50±2.13^{a}	0.003	0.589
71-84d	93.88±5.79	92.34±4.51	88.19±4.55	92.63±3.10	93.23±3.86	0.093	0.719
1-84d	89.26±2.55	87.40±4.37	85.03±3.71	87.80±1.26	86.43±1.61	0.347	0.675

***SEM:** standard error of means. Superscripts (a, b) shows significant differences at each row (P<0.05). The data are presented as mean ± standard deviation*

Table 3. *Effects of GAA addition to diet on EP (%) of laying hens at different periods*

Groups	Negative Control	Positive Control	0.057% GAA	0.114% GAA	0.171% GAA	P	SEM
1-14d	88.04±7.93	82.11±7.77	89.64±3.70	88.21±2.82	87.14±2.95	0.091	0.926
15-28d	86.07±9.12	83.69±5.25	87.23±4.31	86.07±4.41	85.71±4.48	0.656	0.900
29-42d	86.43±8.11	84.94±7.28	86.19±4.68	86.13±6.39	85.60±5.10	0.979	1.12
43-56d	82.32±7.12	82.38±5.03	81.43±2.16	84.46±5.31	87.50±4.56	0.132	0.841
57-70d	84.11±10.23	84.40±4.89	84.59±2.23	83.04±8.16	85.71±4.18	0.952	0.998
71-84d	74.82±12.17	75.45±7.07	74.90±10.18	73.75±9.89	80.18±9.94	0.731	1.53
1-84d	83.63±7.93	82.11±2.35	83.95±2.58	83.61±5.50	85.30±3.63	0.942	0.991

SEM: *standard error of means. The data are presented as mean ± standard deviation*

Table 4. *Effects of GAA addition to diet on EW (g) of laying hens at different periods*

Groups	Negative Control	Positive Control	0.057% GAA	0.114% GAA	0.171% GAA	P	SEM
1-14d	55.60±0.75[a]	55.61±1.09[a]	55.87±0.89[a]	55.14±0.32[a]	53.94±0.80[b]	0.0003	0.164
15-28d	55.80±0.96[a]	55.53±0.77[a]	56.12±0.72[a]	56.34±0.62[a]	54.87±1.18[b]	0.019	0.154
29-42d	56.44±1.02[a]	56.22±1.09[a]	56.47±0.59[a]	56.97±0.84[a]	54.82±1.00[b]	0.0006	0.178
43-56d	56.39±1.42[a]	56.30±1.12[a]	56.85±0.59[a]	56.97±0.76[a]	55.04±0.71[b]	0.003	0.141
57-70d	57.27±1.03[a]	57.57±0.99[a]	57.82±0.71[a]	58.21±0.98[a]	55.16±0.72[b]	0.0009	1.74
71-84d	58.03±1.11[a]	58.03±1.18[a]	57.96±1.36[a]	58.93±0.975[a]	56.80±0.836[b]	0.0124	1.53
1-84d	56.58±0.90[a]	56.54±0.99[a]	56.82±0.57[a]	57.09±0.56[a]	55.26±0.670[b]	0.036	0.209

SEM: *standard error of means. Superscripts (a, b) shows significant differences at each row (P<0.05). The data are presented as mean ± standard deviation*

Table 5. *Effects of GAA addition to diet on EM (g/hen/d) of laying hens at different periods*

Groups	Negative Control	Positive Control	0.057% GAA	0.114% GAA	0.171% GAA	P	SEM
1-14d	48.96±4.69	45.66±2.56	50.08±3.34	48.63±2.67	47.00±3.35	0.469	0.537
15-28d	48.02±5.22	46.47±3.34	48.95±2.75	48.23±2.40	47.02±2.51	0.814	0.400
29-42d	48.78±4.68	47.75±3.10	48.67±4.21	49.06±2.93	46.92±4.85	0.441	0.619
43-56d	46.42±4.04	46.21±2.55	46.29±3.25	48.11±1.45	48.16±2.46	0.446	0.455
57-70d	48.16±5.61	48.58±2.37	48.90±2.53	48.33±1.64	48.13±4.17	0.991	0.536
71-84d	43.41±6.75	43.78±5.31	43.33±3.46	43.46±5.85	45.54±3.14	0.908	0.826
1-84d	47.30±4.40	46.40±1.90	47.70±1.18	47.62±1.84	47.13±2.74	0.982	0.535

SEM: *standard error of means. The data are presented as mean ± standard deviation*

Table 6. *Effects of GAA addition to diet on FCR of laying hens at different periods*

Groups	Negative Control	Positive Control	0.057% GAA	0.114% GAA	0.171% GAA	P	SEM
1-14d	1.79±0.17	1.89±0.25	1.72±0.15	1.77±0.10	1.73±0.10	0.304	0.026
15-28d	1.86±0.20	1.86±0.13	1.77±0.11	1.81±0.15	1.81±0.17	0.398	0.028
29-42d	1.77±0.12	1.85±0.10	1.77±0.10	1.76±0.12	1.75±0.13	0.121	0.038
43-56d	1.87±0.15	1.83±0.12	1.79±0.11	1.78±0.07	1.81±0.09	0.280	0.018
57-70d	1.89±0.23	1.83±0.11	1.72±0.11	1.82±0.14	1.83±0.10	0.656	0.023
71-84d	2.16±0.32	2.10±0.22	2.03±0.31	2.13±0.28	2.04±0.28	0.883	0.044
1-84d	1.88±0.16	1.88±0.07	1.78±0.10	1.84±0.10	1.83±0.10	0.911	0.020

SEM: *standard error of means. The data are presented as mean ± standard deviation*

Table 7. *Effects of GAA addition to diet on reproductive hormones (mIU/mL) and nitric oxide (NO) (μmol/mL) of laying hens at 42 and 84 d*

Groups	Negative control	Positive control	0.057% GAA	0.114% GAA	0.171% GAA	P	SEM
42 d							
LH	5.37±0.24[b]	5.31±0.33[b]	5.44±0.29[b]	6.55±0.83[a]	5.71±0.45[ab]	0.014	0.142
FSH	4.93±0.34[b]	4.84±0.58[b]	5.33±0.25[ab]	5.92±0.70[a]	4.84±0.58[b]	0.041	0.139
NO	216.80±24.72	216.00±14.17	225.00±19.97	272.80±58.09	232.80±17.15	0.113	7.84
84 d							
LH	7.12±0.37[b]	7.52±0.88[b]	7.97±0.42[ab]	8.76±0.43[a]	7.96±0.47[ab]	0.009	0.166
FSH	6.61±0.43[c]	7.06±0.33[b]	7.36±0.21[b]	8.16±0.79[a]	7.10±0.58[b]	0.009	0.155
NO	142.30±56.96	155.80±53.66	153.50±63.61	207.30±33.15	168.10±35.50	0.353	12.49

***SEM:** standard error of means. Superscripts (a-c) shows significant differences at each row (P<0.05). The data are presented as mean ± standard deviation. Each IU LH and FSH is 0.13369 and 0.1138 mg, respectively*

Table 8. *Effects of GAA addition to diet on lipid profile (mg/dL) and enzymes (ng/mL) of laying hens at 42 and 84 d*

Groups	Negative control	Positive control	0.057% GAA	0.114% GAA	0.171% GAA	P	SEM
42 d							
Triglycerides	279.4±23.39	235.10±16.38	224.70±24.00	255.10±19.00	257.30±40.80	0.0749	6.78
HDL-C	40.15±3.73	41.65±2.92	35.83±3.52	41.48±4.68	39.63±6.45	0.381	1.00
LDL-C	84.48±24.72	82.48±14.17	76.60±12.17	84.58±18.09	76.35±17.15	0.685	6.82
AST	175.80±67.31	158.00±66.99	111.50±39.91	147.80±7.50	135.30±34.73	0.445	10.88
ALT	48.25±2.63	51.00±8.90	44.75±3.20	51.00±6.63	53.50±4.93	0.298	1.33
84 d							
Triglycerides	246.70±18.14	280.40±36.47	233.35±57.69	227.00±42.84	242.40±34.66	0.404	9.02
HDL-C	42.70±6.01	42.23±2.93	40.38±6.50	38.60±4.96	37.20±3.53	0.492	1.09
LDL-C	88.25±10.10	94.33±8.76	86.77±17.33	77.25±12.33	79.40±9.40	0.303	3.77
AST	69.75±16.74	64.25±15.84	58.00±15.12	51.75±28.76	56.75±11.59	0.582	4.41
ALT	46.50±5.00	46.75±0.957	46.75±4.99	45.25±3.77	47.75±3.59	0.931	0.805

***SEM:** standard error of means. Superscripts (a-c) shows significant differences at each row (P<0.05). The data are presented as mean ± standard deviation. **AST:** aspartate aminotransferase, **ALT:** alanine aminotransferase*

12th week in laying hens (P<0.05) compared with other groups. Our results are in agreement with the reports of other studies that showed GAA addition to diets had not significant effects on FI of broiler chicks [24,25]. However, Michiels *et al.*[7] observed only slightly increase in FI of broiler chicks fed with GAA. It seems that GAA addition has not significant effect on FI in all birds. FCR was no influenced by GAA treatments and positive control (2% meat meal); showing that creatine supplementing from each source had not significant effect on the mentioned parameter. Tossenberger *et al.*[24] stated that GAA addition to diet had not significant effect on FCR of broiler chicks. Other studies have shown that GAA addition to diet lowered FCR in broiler chicks [2,5,25-27]. Metwally *et al.*[28] showed that GAA addition to diet could spare 50 kcal metabolizable energy and 0.5% crude protein. Ringle *et al.*[25] showed that GAA addition to corn-soybean meal diets may have beneficial effects in improving growth and FCR. Studies have shown beneficial effects of GAA on FCR, but all the studies are conducted in broiler chicks. So far, any study is not conducted to investigate the effects of GAA on productive performance of laying hens. However, exact mechanism of the effects of GAA in broiler chicks is not still known. It is shown that GAA addition to diet has beneficial effects in improving the growth, muscle mass and creatine synthesis [7]. This study was conducted in laying hens and GAA had not significant effect on body changes. Weight changes were by 30 g in all groups in the present study. It seems that GAA induces its effects on FCR by increasing muscle mass and subsequently improves FCR. Increase in body weight in laying hens is not desirable and on the basis our results, GAA has not any role in improving EP, thus GAA cannot increase FCR in laying hens.

The second status was that GAA can spare arginine and help to protein synthesis which finally increases EP and EM. Bryant-Angeloni [29] showed that GAA synthesizes protein by sparing arginine. Considering sparing effect of arginine by GAA, Basiouni *et al.*[30] showed that dietary inclusion of 1.5% digestible arginine to laying hen diet increased production from 52% to 67.86%. Silva *et al.*[31] reported that dietary inclusion of arginine increased EP in broiler breeders. Sparing effect of arginine by GAA may be reason for improving performance in birds which it was found in studies on broiler chicks [29]. In the present study, it seems that GAA could not spare arginine and subsequently did not influence EP. Other status was that GAA addition to diet increases EP by increasing NO which subsequently increases reproductive hormones [12] and the hormones increase EP [15]. In the current study NO was not affected by dietary treatments and EP was not increased. However, an increase in FSH and LH was seen in 0.114 GAA treatment which NO is not responsible for it, because NO levels is constant.

EW was significantly reduced in laying hens fed the 0.171% GAA compared with other groups. In other words, the lowest levels of GAA had not significant effects on EW, while the highest levels reduced it. EW is a parameter which affects by diets protein and amino acid level [32] and the arginine role is accepted as increaser the protein synthesis [33]. The mechanism of action is not known.

Dietary inclusion of 0.114% GAA increased LH and FSH compared with controls at 42 and 84 d. Investigation of relation between nitric oxide and GAA was one of purposes of the present study. It is well known that nitric oxide releases gonadotropin-releasing hormone (GnRH) by activating pituitary nitric oxide synthase which finally influences FSH and LH hormones [12]. It can be found from *Table 7* that nitric oxide was numerically higher in 0.114% GAA group, but the differences were not significant. It seems that nitric oxide cannot be reason for increase in reproductive hormones. Sharifi *et al.*[34] showed that arginine addition to broiler chicks diets significantly increased nitric oxide level. GAA is synthesized from arginine and glycine, it was expected that GAA addition to diet increase nitric oxide level, but such result was not found. It may be attributed to the GAA bioavailability, bird type and age.

Results indicated that GAA addition to diet had not significant effect on blood lipid profiles and liver enzymes. Studies have not still investigated effects of GAA on blood lipid profiles and liver enzymes. Considering studies on arginine, Al-Daraji *et al.*[35] showed that dietary inclusion of arginine reduced the serum concentration of triglyceride in broiler chicks. Blood parameters are important, especially in meat-type birds [36]. Studies have been shown that increase in VLDL is criteria for showing the increased body lipid [37]. Other study reported that the serum concentration of triglyceride is criteria for showing body lipid [36]. In the present study, lipid levels were not influenced by GAA. Nitric oxide is known to have roles in lipid metabolism and thus arginine reduces abdominal lipid and indirectly balances lipid storage by nitric oxide synthesis [18]. In the present study, since nitric oxide level was not affected by GAA supplementing, thus it is reasonable that lipid profile is not also affected. Considering liver enzymes in serum, Yang *et al.*[19] showed that dietary inclusion of arginine significantly reduced levels of liver aspartate aminotransferase in laying hens. The increased aspartate aminotransferase in blood is considered as index for muscle and liver damages, which its levels are different in various birds [38]. The increased alanine amino transferase level is considered as index for tissue damages [38]. Thus, the increased blood levels of these enzymes implicates on tissue and liver damages. In the present investigation, the both levels were not influenced by GAA addition; showing that GAA has not positive or negative effects on liver enzymes.

In conclusion, the present study was conducted to investigate the relation among GAA addition with nitric oxide, reproductive hormones and lipid profile in laying hens. Findings showed that GAA could not improve performance, nitric oxide level and blood biochemical parameters. Dietary inclusion of 0.114% GAA increased LH and FSH. It can be concluded that GAA addition, as a supplement at the used levels, cannot be used for improving performance in laying hens receiving the corn-soybean meal based diets and it cannot appropriate source to sparing arginine at these levels in laying hens. The use of higher levels and also measuring creatine in muscle will be suggested in future studies for understanding relation GAA and creatine in laying hens.

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