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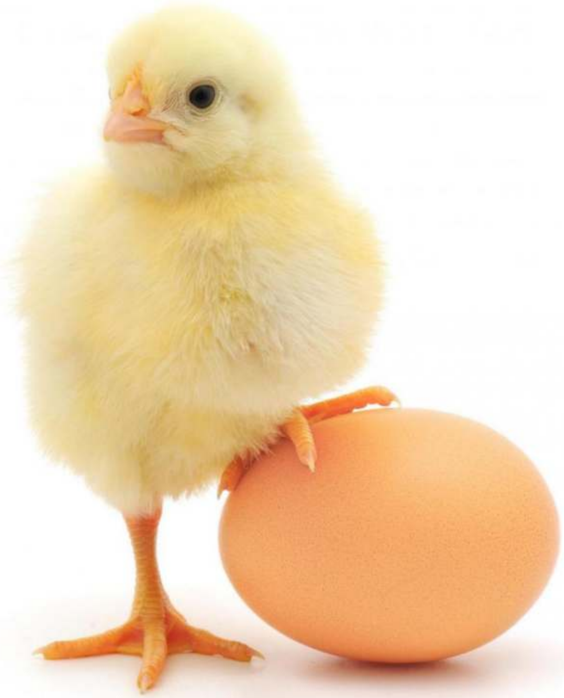

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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Influence of Extra Supplementation with Arginine and Lysine on Overall Performance, Ovarian Activities and Humoral Immune Response in Local Saudi Hens

G. Basiouni[1], H. Najib[1], M.M. Zaki[2] and A.S. Al-Ankari[2]*
[1]Department of Animal and Fishery Production, College of Agricultural Sciences and Food,
[2]Department of Clinical Studies, College of Veterinary Medicine and Animal Resources,
King Faisal University, P. O. Box 55055, Al-Ahsa, 31982, Saudi Arabia

Abstract: This study was designed to investigate the effect of increasing level(s) of arginine (ARG) and/or lysine (LYS) over the normal requirements of the Leghorn breeds (NRC, 1994) and their interaction on performance, ovarian activity and humoral immune response of local Saudi chickens known for their low production. The outcome of the study revealed that feed intake and specific gravity of the eggs were the only traits significantly (P<0.05) affected by the interaction between ARG and LYS. Hen-day production did not significantly (P>0.05) increase and feed conversion improved with the higher levels of ARG and LYS (1.54 and 2.05 %, respectively) compared to the controls. In spite of the significant effect of specific gravities of the eggs, no specific trend was found in response to the treatments. Albumen height was better in the control group in both treatments which supported the higher egg size of the control group compared to the other treatments. No significant differences were found among treatments or their interaction in all ovarian and oviductal measurements. However there was a tendency for an increase in the weight of F1 follicles (largest follicles) as a result of ARG supplementation at the 2.04 % level. The study of humoral immune response of the birds revealed that mean antibodies titers (MAT) at week 24, 28, 32 and 40 of age were nearly the same in all groups of birds and no significant differences between the groups in the designated periods (P>0.05). However, at week 36 of age, MAT of group treated with 1.54% ARG and 1.05% LYS were significantly higher than the controls (P<0.05). A significant correlation between MAT and thymus weight index at week 40 of age was observed. Based on the results of this study, it is concluded that extra ARG, LYS and their interaction did not significantly affect performance and ovarian activity of the local birds but enhanced thymus size and maintain MAT level over time.

Key words: Arginine, lysine, local Saudi chickens, ovarian activity, immunity

Introduction

Arginine (ARG) is known for its role to enhance the ovulation process of the hens through increasing release of luteinizing hormone (LH) which is necessary for ovulation in both, farm animals and chickens. It also participates in multiple biological processes including antibacterial activities and non-specific immunity (Potenza *et al.*, 2001).

ARG was found to stimulate LH release in pre-pubertal ewes (Recabarren *et al.*, 1996a and 1996b) and goats (Basiouni *et al.*, 1999). Measurement of such ovarian weight, oviductal and follicle size was used to examine the effect of hormonal secretion pattern on the ovary (Liu *et al.*, 2002; Lovell *et al.*, 2001). Takahashi *et al.* (1994) reported that ARG, as part of the hormone arginine vasotocin, (neurohypophysial hormone) plays an important role in the initial contraction of hen's uterus through an increased binding to its receptor which might affect the process of oviposition in hen.

Importance of Lysine (LYS) as the second limiting amino acid for the leghorn layer and for its' antagonism with ARG has been documented (Leeson and Summers, 2001; Scott *et al.,* 1982; D'Mello and Lewis, 1970).

Local Saudi chickens have been known for their poor performance (Al-Aqil, 1998). Al-Yousef *et al.*, (1999) found an average egg production ranged between 37-52% when the local breed fed levels of ARG and LYS in the diet as recommended by (NRC, 1994). Later, Najib and Basiouni, (2004) improved level of egg production of the breed by 67.86 % as levels of ARG and LYS were increased in diet to 1.5 and 1.2%, respectively. The authors concluded that local Saudi chickens might have the potential to perform better as a level of some amino acid risen up and suggested that further investigation is necessary to determine the best ARG/LYS combination levels.

Lymphoid organ size and development have been known to be directly correlated with the health status of animals. In mammal, dietary ARG improves thymus weight and function (Efron and Brbul, 1998; Bistrain, 2004). In chickens, ARG deficiency has been associated with poor development of thymus and spleen (Kwak *et al.*, 1999), thymus size being a sensitive indicator of health and of acute and chronic stress response (Shelat *et al.*, 1997).

Therefore, this study was carried out to determine the effect of increasing ARG level beyond that suggested by Najib and Basiouni (2004) and to determine the best combination levels of ARG / LYS on the performance, ovarian activities and immune response in the local Saudi layers.

Materials and Methods

During the starting period (0-4 weeks of age), 250 female chicks were placed in a colony metal cages and during the growing period, the chicks were transferred randomly to grower cages (10 per cage). After the chicks' arrival, they were weighed by groups and fed commercial starter diets, containing 21 % crude protein and 2900 Kcal/Kg metabolizable energy (ME). The chicks were kept on the starter diet till 4 weeks of age after which they were switched to the commercial grower diets. These were vaccinated against the most prevailing diseases of Al-Ahsa area.

Chicks were exposed to continuous lighting during the first week of their life then decreased by two hours weekly till reached 8 hours, at which time lighting was held constant. Debeaking was carried out at 4 weeks of age and was done again on week 16 of age.

A 2 × 4 factorial design was used, where two levels of ARG 1.54 and 2.05 % were used in combination with three levels of LYS 1.05, 1.67, 2.34 % and a control. This resulted into 8 dietary treatments. Each treatment was replicated 6 times [cages (50 × 47 cm), each containing 4 birds]. The control diet contains no extra ARG or LYS. The rest of the diets were formulated to contain ARG and LYS as in the control diet plus added amount of either one to make the required levels of each treatment. These diets are shown in Table, 1 and 2.

During the production period (experimental period), feed consumption and egg production were recorded daily. Feed efficiency (Kg feed/egg mass) was calculated by using the average feed intake per hen divided by egg mass (% egg production × egg weight). Egg weight, albumen height and specific gravity were determined on weekly basis on two days collection. Specific gravity method was used to measure the shell quality of the eggs, as described by North, (1984).

Daily feed was given *ad-libitum* and feed left was weighed at the end of each week to determine feed intake. The feeding trial continued for 20 weeks.

Ovarian and oviductal activities: Measurements of the ovarian activities were taken from 64 hens (8/treatment) on four consecutive days on week 39 and 40 of age (19 and 20 weeks of production). Hens were slaughtered and the following measurements were taken at necropsy: body weight (BW), ovarian weight (OVRWT), oviductal weight (OVIWT) without yolky follicles, total number and weight of each hierarchical yolky follicle (follicles = 0.50 g).

Evaluation of the immune response of the birds

Titration of antibodies against Newcastle disease virus vaccine: Blood was drawn from brachial veins of 4 birds of each group at weeks 24, 28, 32, 36 and 40 of age, serum was separated and stored at -20°C until used. Antibodies against Newcastle disease virus (NDV) was measured using ELISA technique described by Snyder *et al.* (1984). The ELISA Kit was obtained from Synbiotics Corporation (Via Frontera, San Diego, CA, USA). Results were calculated and expressed as $\log_{10}$ (antibody titers).

Lymphoid organ weight index (LOWI): Eight birds from each group were weighed individually and then necropsied at 40 weeks of age. The spleen, thymus and harderian glands (all lobes) were immediately removed, carefully stripped of adhering connective tissues and weighed individually. LOWI was calculated for each organ according to the following equation (Konashi *et al.*, 2000):

$$LOWI = \text{Organ weight (gm)} / \text{live body weight (gm)} \times 1000.$$

Statistical analysis: Analysis of variance (ANOVA) for productive traits and ovarian activities measurements were performed by proc mixed procedure (SAS Institute, 1986) for a completely randomized design with 2 × 4 factorial arrangements. The following model was used to determine the differences between treatment groups

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}\text{, where;}$$

Y_{ijk} = variable measured, μ = overall mean, α_i = effect as a result of the i^{th} intake of ARG, β_j = effect of j^{th} intake of LYS, $(\alpha\beta)_{ij}$ = interaction effect of the i^{th} level of ARG and j^{th} level of LYS, ϵ_{ijk} = error components. Means differences were detected by Duncan Multiple Range Test (Duncan, 1955).

ANOVA of repeated measures was conducted with the immune response data using SPSS 12.0 (SPSS, Chicago, IL, USA). Comparison of means in different groups was made by Duncan's multiple range tests (Duncan, 1955). Correlation coefficient was employed to examine the association between means antibody titers in different groups and the lymphoid organ weight index. $P<0.05$ was accepted as statistically significant.

Results and Discussion

Performance: Results of the chicken's performance as affected by increasing levels of ARG and LYS are presented in Tables 3 and 4. It was clear that with exception of feed intake and specific gravity of the eggs, no significant interaction was observed on the production parameters.

Although there was a significant interaction between ARG and LYS in feed intake (Table 3), no specific trend

Table 1: The ingredients used in the basal and treatment diets

Ingredients %	Basal	1.5 ARG+ 1.0 LYS	1.5 ARG+ 1.5 LYS	1.5 ARG+ 2.0 LYS	2.0 ARG+ 1.0 LYS	2.0 ARG+ 1.5 LYS	2.0 ARG+ 2.0 LYS
Yellow Corn	60.43	59.82	60.14	60.46	58.78	59.11	59.43
SBM (Soybean Meal), 44 %	28.04	28.12	26.81	25.5	28.29	26.98	25.66
Choline Cl, 60 %	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Salt	0.29	0.29	0.29	0.29	0.29	0.29	0.29
DL-Methionine	0.08	0.08	0.08	0.09	0.08	0.09	0.09
DCP (Dicalcium phosphate), 19 %	1.4	1.4	1.44	1.48	1.4	1.44	1.49
Limestone	8.13	8.13	8.12	8.1	8.13	8.11	8.09
Oil	1.25	1.47	1.71	1.96	1.83	2.07	2.32
Carophyll	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Breeder premix[1] 0.2 %	0.2	0.2	0.2	0.2	0.2	0.2	0.2
L-LYS, added	0	0.02	0.68	1.35	0.01	0.68	1.35
L-ARG, added	0	0.3	0.35	0.39	0.81	0.85	0.9
Total	100	100	100	100	100	100	100

[1]The multi vitamin minerals premix provided the following per kilogram of diet; 7,000,000 IU vitamin A, 1,500,000 ICU vitamin D_3, 30,000 IU vitamin E, 50,000 mg vitamin C, 2,300 mg vitamin C, 1,400 mg vitamin B_1, 5,520 mg vitamin B_2, 2,300 mg vitamin B_6, 12 mg vitamin B_{12}, 27,600 mg Niacin, 920 mg folic Acid, 9,600 mg pantothenic acid, 92 mg biotin, 50,000 mg antioxidant (BHT), 220 mg cobalt, 4,400 mg copper, 800 mg iodine, 26,400 mg iron, 44,000 mg manganese, 180 mg selenium, 44,000 mg zinc

Table 2: The calculated composition of the basal and experimental diets

Ingredients %	Basal	1.5 ARG + 1.0 LYS	1.5 ARG + 1.5 LYS	1.5 ARG + 2.0 LYS	2.0 ARG + 1.0 LYS	2.0 ARG + 1.5 LYS	2.0 ARG+ 2.0 LYS
DM	89.82	89.58	89.62	89.67	89.18	89.22	89.26
PROTEIN, %	18	18	18	18	18	18	18
ME, Kcal/Kg	2800	2800	2800	2800	2800	2800	2800
Fat, %	3.92	4.12	4.34	4.58	4.44	4.66	4.9
FIBER, %	2.25	2.24	2.2	2.15	2.22	2.18	2.14
ASH, %	12.11	12.12	12.07	12.01	12.11	12.06	12.01
CALICIUM, %	3.51	3.51	3.51	3.51	3.51	3.51	3.51
PHOS, TOT., %	0.6	0.6	0.6	0.6	0.6	0.6	0.6
PHOS. AV., %	0.31	0.31	0.31	0.32	0.31	0.31	0.32
NaCl, %	0.35	0.35	0.35	0.34	0.35	0.35	0.34
Na, %	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Lysine,%[1]	0.99	1.01	1.67	2.34	1.21	1.67	2.34
ARG, %[2]	1.2	1.5	1.55	1.59	2.01	2.06	2.1
Methionine, %	0.38	0.38	0.38	0.38	0.38	0.38	0.38
METH+CYS., %	0.72	0.72	0.7	0.69	0.72	0.71	0.69
CHOLINE, %	1382.1	1382	1347.2	1312.6	1382	1347.5	1312.7

[1],[2] Lysine and Arginine levels after supplementation

was found. With the exception of 1.54% ARG 2.34% LYS treatments, control group consumed more feed than other treatments.

Birds fed 1.54 % ARG consumed more feed as level of LYS increased from 1.05 to 2.34% (Fig. 1). Opposing those, birds that fed 2.05 % ARG lowered their feed consumption as level of LYS increased from 1.05% to 1.67%. Optimum consumption was found in birds fed 1.54 ARG/1.5 LYS. Prochaska *et al*. (1996) reported that feed consumption was significantly reduced in hens consuming the highest dietary LYS (1,165 mg/hen/day) compared with other 3 treatments which were similar.

The higher consumption of birds fed 1.54 % ARG/2.34 % LYS could have been due to the LYS/ARG antagonism phenomenon. Limited increase of LYS level in a diet can cause marked elevation of arginase activity consequently increased ARG degradation which may necessitates the increase in ARG level in the diet (Scott *et al*., 1982). Therefore, it could be possible that the birds had tried to eat more in an attempt to compensate for the differences in ARG level.

It was unclear why would 1.54 % ARG along with 1.05 % and 2.34 % LYS gave the best specific gravities while when 2.05 % ARG combined with 2.34 % LYS had the worst result. These findings were very much similar to the data presented by Najib and Basiouni (2004) regarding the unharmonious response of specific gravity to the ARG levels. Novak *et al*. (2004) found no differences between LYS level related to specific gravity of the eggs in dekalb hens. With the exception of (2.05 ARG:2.34 LYS), performance of other combinations may seem excellent with an average of 1.090 specific gravity which have been due to the smaller size of the local Saudi chickens eggs.

Shell synthesis in birds is constant but the decreased egg size would have caused the proportion of shell per egg to increase.

The effect of ARG on hen-day production was not

Table 3: The effect of ARG / LYS interaction on production traits of the local Saudi chickens.

TRT --------------------------- ARG %	LYS %	FI (g/b/d)	FC (Kg/Kg)	EW (g)	HD (%)	EM (g/HD)	SPG	AH (mm)
Control		100.5±14.7	4.014±1.3	43.85±3.9	61.64±16.5	27.40±9.0	1.090±0.004	4.91±1.58
2.05	1.05	95.8±20.1	3.764±1.6	43.53±3.0	64.93±19.9	28.29±8.9	1.091±0.004	4.57±0.62
2.05	1.67	92.2±13.8	3.782±1.5	42.80±3.3	62.04±19.7	26.84±7.7	1.092±0.004	4.79±0.64
2.05	2.34	93.2±19.2	3.768±1.6	42.81±3.3	64.78±19.4	27.75±8.5	1.089±0.004	4.70±0.64
1.54	1.05	89.7±21.8	3.544±1.3	44.03±4.2	62.66±19.3	27.64±8.7	1.092±0.004	4.79±0.73
1.54	1.67	93.6±22.1	3.678±1.3	43.43±3.8	64.41±17.7	27.93±7.7	1.090±0.004	4.60±0.85
1.54	2.34	100.4±22.0	4.094±1.5	41.97±3.3	64.84±20.7	27.18±8.9	1.092±0.004	4.53±0.71
P =		0.0015**	0.1433NS	0.1019 NS	0.5158 NS	0.6112 NS	0.0001**	0.0812NS

ARG= % ARG; LYS = % LYS ; FI = gram per bird per day, feed intake ; FC = kg feed per kg eggs, Feed conversion; EW = g, egg weight; HD = hen-day production, % ; EM = % HD * egg weight; SPG = specific gravity of the eggs ; AH = albumen height, mm.
*significant difference at level of (P<0.05), **significant difference at level of (P<0.001), NS no significant difference.

Table 4: The effect of incorporating extra amount of ARG and LYS on productivity of the local Saudi chickens

TRT	FI (g)	FC (Kg/Kg)	EW (g)	HD (%)	EM (g/HD)	SPG	AH (mm)
ARG	NS	NS	NS	NS	NS	*	NS
1.54	94.39[b]	3.769[a]	43.16[b]	63.85[a]	27.55[a]	1.0914[b]	4.653[b]
2.05	94.01[b]	3.770[a]	43.11[b]	64.19[a]	27.77[a]	1.0903[a]	4.669[b]
P =	0.5875	0.9949	0.7571	0.975	0.9514	0.0734	0.5874
LYS	*	NS	**	NS	NS	NS	NS
Control	100.54[a]	4.014[a]	43.85[a]	61.64[a]	27.40[a]	1.0903[a]	4.908[a]
1.05	92.78[b]	3.656[b]	43.78[ab]	63.82[a]	27.97[a]	1.0913[b]	4.677[b]
1.67	92.92[b]	3.729[ab]	43.12[ab]	63.23[a]	27.39[a]	1.0909[ab]	4.692[b]
2.34	96.82[ab]	3.933[ab]	42.38[b]	64.81[a]	27.46[a]	1.0905[ab]	4.619[b]
P =	0.0598	0.1418	0.0005	0.736	0.7713	0.1258	0.7575

Means in columns that are not followed with the same superscripts are significantly different, P<0.05. *significant at 10 % of probability, **significant at 1 % of probability, NS not significant; EW = g, egg weight; ARG = % ARG; LYS = % LYS; FI = gram per bird per day, feed intake; FC = kg feed per kg; eggs, Feed conversion; HD = hen-day production, %; EM = % HD * Egg Weight; SPG = specific gravity of the eggs; AH = albumen height, mm.

significant at 5 % level of probability (Table 4); however, there was a clear trend that as level of ARG increased from zero supplementation (basal diet) to 1.54 and 2 .05 % ARG in the diet has improved the production by about 4 %.

Najib and Basiouni (2004) found similar results when 1.54 % ARG was included in the diets of local Saudi chickens. These two studies, with no doubt, provided strong evidence that increased ARG dietary level over the level of what the leghorn needs would be necessary to optimize production in the local Saudi chickens. Cheng *et al.* (1999) studied the requirements of local Beijing brown hens and found that, when LYS was assumed to be 100, ARG proportion was found to be 100 also.

Increased dietary level of LYS, in this experiment, was found to be beneficial in obtaining extra production especially at the high levels. However, the differences were not significant (P>0.05) and there was at least 3% of extra production over the control. Prochaska *et al.* (1996) reported that overall feed intake and egg production were not affected by increasing dietary LYS from 677 to 1154 or 1613 mg/hen per day. Likewise, Novak *et al.* (2004) found that egg production and feed consumption were not affected by increasing dietary LYS in Dekalb Delta laying hens. Novak and Scheideler (1998) and Scheideler *et al.* (1996) reported that the current LYS requirement (680 mg/hen/day) listed in the NRC (1994) may be substantially lower than what is required for optimal egg mass, feed efficiency and egg production. The results of these authors agreed with the assumption of this experiment that some amino acids may be under estimated in the NRC especially under harsh experiment and if this was true for the white leghorn then local Saudi chickens may be deficient in some amino acids as well.

In this experiment, extra ARG had no significant effect on egg weight (P=0.7571) (Table 4). Najib and Basiouni (2004) found no significant effect of feeding different levels of ARG ranging from 0 to 2.5 % on average egg weight in their first experiment. The addition of 2.34 % LYS significantly depressed the egg weight of the birds compared to controls. No significant differences were observed between the controls, 1.05 and 1.67 % LYS.

Novak *et al.* (2004) reported an increase of egg weight from 59.02 to 60.21 g with increasing LYS from 860 to 959 mg/hen/day in Dekalb laying hens and attributed that to the increase in albumen weight. Albumen weight was not measured in this experiment, however, Albumen height, measured in millimeter was the largest in eggs of the control group in both ARG and LYS fed birds and incidentally had the largest egg weight. No significant differences were observed among ARG or LYS levels and no trend was noticed in egg mass in this study. Similar results were reported by Najib and Basiouni (2004).

Table 5: Effect of ARG, LYS levels on ovarian and oviductal activities of the local Saudi chickens[1]

TRT	BW	OVRWT	OVIWT	F1WT	F2WT	HRFO
ARG, (%)	NS	NS	NS	*	NS	NS
1.54	1345.5[a]	7.78[a]	39.14[a]	12.58[a]	9.41[a]	4.97[a]
2.05	1359.2[a]	7.15[a]	40.80[a]	13.14[b]	9.88[a]	4.97[a]
P =	0.7231	0.5285	0.8603	0.0947	0.2563	1.0000
LYS, %	NS	NS	NS	NS	NS	NS
0.0	1340.6[a]	7.55[a]	39.47[a]	12.70[a]	9.28[a]	4.75[a]
1.05	1380.6[a]	6.93[a]	40.58[a]	13.28[a]	10.01[a]	5.00[a]
1.67	1364.4[a]	8.79[a]	41.26[a]	12.88[a]	9.56[a]	4.94[a]
2.34	1323.8[a]	6.58[a]	38.58[a]	12.60[a]	9.73[a]	5.19[a]
P =	0.7361	0.4261	0.7686	0.4889	0.6533	0.3893

[1] Means that are not followed with same superscript are significantly different, p<0.05; NS = Not significant, P>0.05; * = significant at 10% of probability. TRT = treatments; ARG = ARG level; LYS = LYS level; BW = body weight, g; OVRWT = ovary weight, g; OVIWT = oviduct weight, g; F1WT = largest follicle Weight, g; F2WT = second to largest follicle weight, g; HRFO = No. of hierarchy follicles, (= 0.50 g).

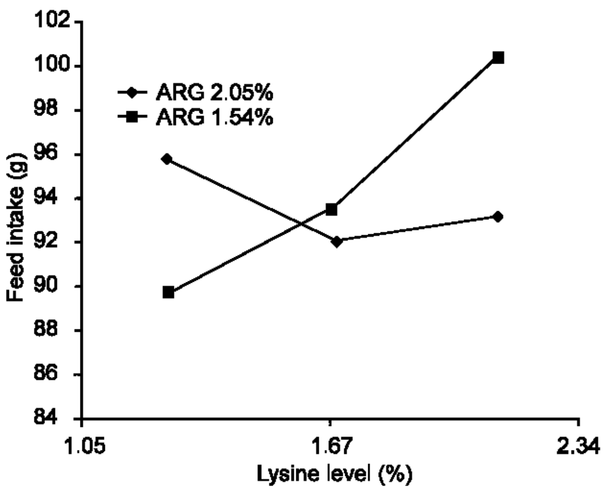

Fig. 1: The effect of Arginine/lysine interaction on feed intake in local Saudi chickens

Ovarian and oviductal activities: No significant differences were found among treatments or their interaction in all ovarian and oviductal measurements taken (Table 5). Also, there was an insignificant (P = 0.0947) increase in F1 follicles weight in chickens fed 2.05 % ARG compared to 1.54 % ARG fed chickens (13.14g and 12.58 g, respectively). This numerical difference may indicate a tendency for an increase in follicular maturation as a result of ARG supplementation at the 2.05 % level.

It appears that ARG may affect egg production through mechanism(s) other than acting directly on the ovary and / or its follicles. In turkey hens, Liu *et al.* (2002) found that both, higher plasma LH concentrations and shorter preovulatory LH surge intervals were associated with higher rate of egg production.

In the present study, it is possible, therefore, that the significant increase in egg production as a result of feeding an extra amounts of ARG may be due to its specific stimulatory effect on LH secretion and ovulation rather than acting directly on the ovary and its follicle

Effect of ARG/LYS diet on the immune system

Effect on Humoral Immune Response to NDV Live Vaccine: Birds of all groups were vaccinated against Newcastle disease virus at weeks; 2, 4, 7, 9, 12, 18 and 34 of age. Mean antibody titer (MAT) among groups was shown in Fig. 2. MAT at week 24, 28, 32 and 40 of age showed almost constant trend with slight variations and no significant differences between the groups in the designated periods (P>0.05). However, MAT reduction was observed in the control group starting from week 32 and a significant difference between MAT in the controls (3.89±0.03) and group 2 (4.15±0.03) was observed at week 36 of age (P<0.05).

Such reduction is expected under normal condition as a result of the long duration between the two vaccination doses that were given at week 18 and week 34. However, groups received ARG/LYS in their diet, did not show such reduction and nearly maintain constant trend until week 36. This may suggest that extra ARG/LYS maintains a proper immune function over time.

ARG has been reported to play an important role as a potent immunological modulator through production of nitric oxide (Collier and Vallance, 1989) and has been shown to directly influence the immune system of birds under several experimental models (Kidd *et al.*, 2001; Friedman *et al.*, 1998).

The outcome of this study is in accordance with the findings of Kidd *et al.* (2001) and Shang *et al.* (2003) who found ARG supplementation may enhance humoral immunity and attenuate the oxidative stress induced by burns in mice.

Corzo and Kidd (2003) indicated that chicks have a considerable critical need for dietary ARG at an early age which possibly was associated with immune system development and early microbial challenges and, in fact, Abdukalykova and Ruiz-Feria (2006) demonstrated that high level of ARG can accelerate antibody production in broiler chickens. Further study by Miller (2004) indicated that thymus could carry out the function performed by the bursa of Fabricius in chickens and he believed that the only thymus-derived lymphocytes could be involved in the antibody production.

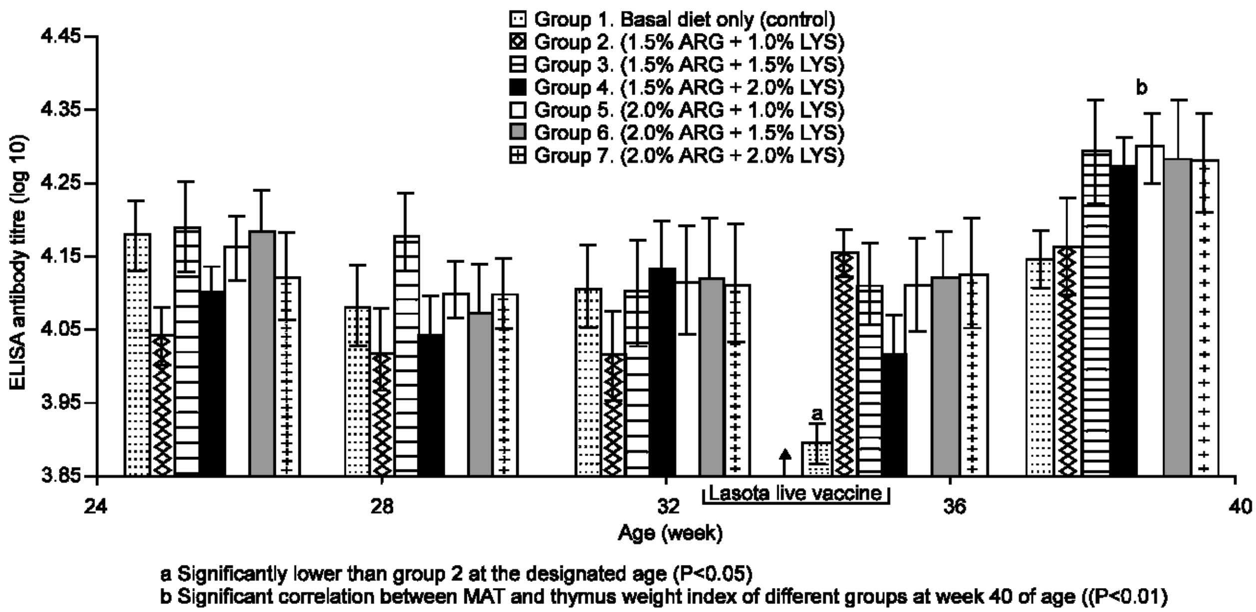

Fig. 2: Antibody titres (mean ±SD) against Newcastle Disease Virus (NDV) in local Saudi chickens fed basal diets supplemented with different levels of ARG.

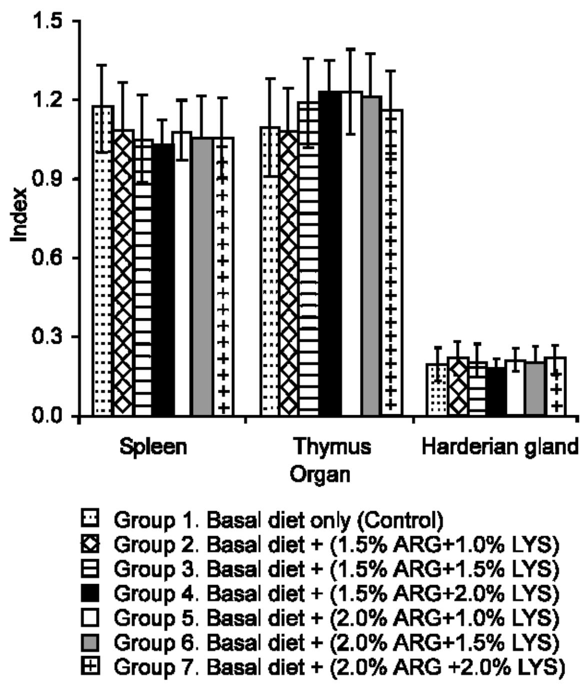

Fig. 3: Lymphoid organ index of spleen, thymus and harderian glands of local Saudi chickens fed basal diet supplemented with different level of ARG

There were no significant MAT differences between the controls or group 2 and the other groups at the designated age. At week 40, MAT increased as a result of a booster dose of vaccine for all groups of the study.

Effect on LOWI: The relative weight index of spleen, thymus and harderian gland are shown in Fig. 3. Generally, ARG/LYS levels did not significantly affect the relative size of any of the three lymphoid organs (P>0.05). However, groups 2, 3, 4, 5, 6 and 7 had numerically heavier thymus than the controls and significant correlation between thymus weight index (r = 0.0907; P = 0.005) and MAT at week 40 was observed indicating that the size of thymus is a good indicator of immune function (Fig. 2 and Fig. 3).

Such observation was supported by the findings of Kwak *et al.* (1999) who indicated that ARG deficiency has been associated with poor development of thymus in chickens. Also, Shelat *et al.* (1997) revealed that thymus size is a sensitive indicator of health and of acute and chronic stress response. Other studies by Popova-Ralcheva *et al.* (1998) concluded that enrichment of the balanced diet with 1 and 2% ARG maintained relative weight of thymus and also, Efron and Brbul (1998) revealed that the dietary ARG improves thymus weight and function in mammals.

Although, spleen weight index (r = -0.729; P = 0.063) and harderian gland weight index (r = 0.090; P = 0.848) did not significantly correlated with MAT at week 40 (P>0.05), Kwak *et al.* (1999) concluded ARG deficiency has been associated with poor development of spleen. The authors do not have instant explanation for the contradiction between our result and the conclusion of Kwak *et al.* (1999).

Many factors could be attributed to such contradiction; these could be due to genetic properties of the local breed or to small number of birds used for calculation LOWI. Further study using larger sample size is worth considering.

Conclusion: In conclusion, data of egg production traits provided evidences to suggest that feeding higher ARG and LYS dietary levels beyond that suggested by Najib and Basiouni, (2004). (1.2% and 1.5% for LYS and ARG) did not significantly improve production traits, ovarian and oviductal measurements in the local Saudi chickens. Further studies are needed to re-evaluate the other amino acids requirements for the local Saudi chickens, namely methionine, cystine, threonine tryptophan, leucine and isoleucine. However, increasing ARG/LYS levels beyond that suggested by Najib and Basiouni, (2004) maintained a proper immune function for the local birds over time.

The effect of LYS supplementation on LH secretion in relation to egg production needs to be examined also.

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