



## EFFECTS OF ARGININE AND VITAMIN E SUPPLEMENTED DIETS ON THE IMMUNOLOGICAL RESPONSE OF BROILERS CHICKENS

[EFECTO DE DIETAS SUPLEMENTADAS CON ARGININA Y VITAMINA E EN LA RESPUESTA INMUNOLOGICA DE POLLOS DE ENGORDA]

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### SUMMARY

In order to evaluate the effect of arginine and vitamin E supplementation in broiler chicken diets on the immune response during post-vaccine stress, a trial was conducted with 700 chicks (1 day-old) which were distributed into 28 floor-pens and fed one of four dietary treatments (with 7 replicates) assigned randomly: T1 = control diet (1.31 % of arginine and 10 IU of vitamin E/kg of feed); T2 = T1 + 0.3 % of arginine; T3 = T1 + 70 IU of vitamin E; T4 = T1 + 0.3 % of arginine + 70 IU of vitamin E. At day 12 all birds were vaccinated against Newcastle disease virus (NDV), infectious bronchitis, avian influenza (AI) and fowl pox. The traits evaluated were: post-vaccine reaction at days 14, 16, 18, 21 and 23; antibody titers against NDV and AI, and relative lymphoid organs weight at days 11, 19 and 26; and the performance were recorded weekly. Chickens fed T2, T4 (at day 16), and T3 (at day 21) had lesser ( $p \leq 0.05$ ) post-vaccine reaction than birds fed T1. The antibody titers against NDV (at day 11) was higher ( $p \leq 0.05$ ) in chickens fed T4 (3.1 log<sub>2</sub>), T3 (2.7 log<sub>2</sub>) and T2 (2.7 log<sub>2</sub>) compared to T1 (1.6 log<sub>2</sub>); meanwhile, for AI titers no differences were found. There were no differences, neither for relative immune organs weight, nor for performance. In conclusion, arginine and vitamin E supplementation in broiler chickens

diets reduced the post-vaccine stress and improved the immune response without affecting the performance.

**Key words:** chicken, immunological response, post-vaccine reaction, vitamin E, arginine.

### RESUMEN

Para evaluar el efecto de suplementar arginina y vitamina E en la dieta de pollos de engorda en la respuesta inmune durante estrés postvacunal, se realizó este experimento con 700 pollos, de un día de edad, los cuales fueron distribuidos en 28 corrales y alimentados con uno de cuatro dietas experimentales (con 7 repeticiones) asignadas aleatoriamente: T1 = dieta testigo (1.31 % de arginina y 10 UI de vitamina E/kg de alimento); T2 = T1 + 0.3 % de arginina; T3 = T1 + 70 UI de vitamina E; T4 = T1 + 0.3 % de arginina + 70 UI de vitamina E. A los 12 días de edad, los pollos fueron vacunados contra la enfermedad de Newcastle (EN), bronquitis infecciosa, influenza aviar (IA) y viruela. Se evaluaron las variables: reacción postvacunal, a los 14, 16, 18, 21 y 23 días; título de anticuerpos contra EN e IA, y peso relativo de los órganos linfoides, a los 11, 19 y 26 días; y semanalmente se registró la producción. Los

pollos alimentados con T2, T4 (16 días), y T3 (21 días) tuvieron menor ( $p \leq 0.05$ ) reacción postvacunal comparados con T1. El título de anticuerpos contra EN (11 días) fue mayor ( $p \leq 0.05$ ) en pollos alimentados con T4 (3.1 log<sub>2</sub>), T3 (2.7 log<sub>2</sub>), y T2 (2.7 log<sub>2</sub>) contra T1 (1.6 log<sub>2</sub>); el título contra IA no fue diferente. No hubo diferencias en peso de órganos

linfoides, ni en producción. En conclusión, la suplementación con arginina y vitamina E en la dieta de pollos reduce el estrés postvacunal y modula la respuesta inmune, sin alterar la producción.

**Palabras clave:** pollos, respuesta inmunológica, reacción postvacunal, arginina, vitamina E.

## INTRODUCTION

In the poultry production systems in addition to physical bio-security measures there are also immunological procedures, which are applied through strict vaccination programs, both with the aim to reduce the incidence and consequences of the infectious agents, such as virus, bacteria and molds (Martinez and Sanz, 2006). However, although the objective of the vaccination is to produce an immunological response to avoid the infection (Al-Garib *et al.*, 2003), some vaccines, based on live virus, can cause immunosuppression in poultry with the consequent emergence of postvaccinal reactions such as sneeze, sudden movements of head, tearing and eye inflammation (Perozo *et al.*, 2004), these postvaccinal reactions predispose the chickens to secondary bacterial infections (Butcher and Heskett, 2010).

Because the immune system is mediated by production of chemical compounds of protein origin, such as: antibodies, immunoglobulins and cytokines, this is dependent on the availability of nutrients during an excessive stimulation (Li *et al.*, 2007). Therefore, nutritional regimes that increased the immunity, reducing the severity of the diseases must be evaluated (Field *et al.*, 2002; Kidd, 2004).

Arginine and vitamin E are nutrients with potential to modulate the immune system. The vitamin E supplementation in broiler chickens diets, in moderate quantities (25 to 50 IU / kg of feed), increase the production of antibodies against infectious bronchitis, module the free radicals and antioxidants production, and mediate the lymphocyte population dynamics during an infection with lypopolysaccharides of *Salmonella typhimurium* (Leshchinsky and Klasing, 2001, 2003).

On the other hand, it has been shown that L-arginine supplementation modulates the immune response (Kwak *et al.*, 2001; Jahanian, 2009; Munir *et al.*, 2009), probably through two metabolic ways: first, by

the arginase way, where arginine is degraded and favors the polyamines and proline synthesis. Polyamines are related to the lymphocytes proliferation; meanwhile, proline is associated with the collagen synthesis to heal damaged tissues (Fernandes and Murakami, 2010). The second way is the nitric oxide synthesis by the action of the inducible nitric oxide synthase; this way is essential to the cytotoxic activity of macrophages and also stimulates the vasodilatation to repair tissues (Le Floc'h *et al.*, 2004).

Also, it has been investigated the effect of the concurrent supplementation of arginine and vitamin E on the immune system of broiler chickens. These results suggest a complementary role on the humoral and cellular immune function (Abdukalykova *et al.*, 2008; Ruiz-Feria and Abdukalykova, 2009; Perez-Carbajal *et al.*, 2010).

Finally, to produce broiler chickens at least cost it is necessary: to use at maximum the genetic potential and prevent efficiently the diseases, among others. Unfortunately, the nutrient requirements to maximize the body weight gain are not the same for best immune response (Jahanian, 2009); thus, it is necessary to investigate the nutrient levels that improve the avian immune system, and, as a result, avoid the reduction in performance due to stress by diseases.

By the reasons mentioned previously, it was evaluated the effects of the supplemental vitamin E, arginine or both, in broiler chicken on: a) the postvaccinal reaction expressed by respiratory noise, sudden movements of head and tearing; b) the weight of lymphoid organs (thymus, spleen and bursa of Fabricius); c) the humoral immunity, assessed as the antibody titers against Newcastle disease and Avian Influenza; d) performance traits, assessed as the body weight gain, feed intake, feed conversion ratio and mortality

## MATERIAL AND METHODS

### Experimental diets

The experimental diets were: T1 = control diet (1.31 % of arginine and 10 IU of vitamin E/kg feed); T2 = control diet + supplemental 0.3 % of arginine (1.61 % of arginine and 10 IU vitamin E/kg feed); T3 = control diet + supplemental 70 IU vitamin E (1.31 % of arginine and 80 IU vitamin E/kg of feed); T4 = control diet + supplemental 0.3 % of arginine and 70 IU vitamin E (1.61% of arginine and 80 IU vitamin E/kg of feed). Feed was corn-soybean meal based and formulated free of coccidiostat or antibiotics, and according to the nutrient requirements indicated by Lemme *et al.* (2004; Table 1).

### Animal management

Broiler chickens (n = 700), one day old, of the commercial line Ross 308 were grown in floor pens with oat hulls as litter during four weeks. The four experimental diets, with seven replicates each, were randomly distributed to 28 pens of 25 chicks each one. Chickens had free access to feed and water during the entire trial period.

At 12 days old, all chickens were vaccinated against Newcastle disease, Avian Influenza, infectious Bronchitis and fowl pox. For Newcastle, the commercial strain La Sota was applied by eye drop vaccination in combination with the Infectious bronchitis vaccine, serotype Massachusetts. The mineral oil emulsion of Avian Influenza subtype H5N2, strain A/chickenMéxico/232/CPA/94 of low pathogenicity was applied subcutaneously, and the fowl pox vaccine (Gibbs strain) was applied subcutaneously by wing-web puncture.

### Postvaccinal reaction

All chickens of each pen were monitored during three minutes at 14, 16, 18, 21 and 23 days old, and the presence of the following were recorded: respiratory noise, sudden movements of head and tearing. To assess the post vaccine reaction the method described by Torres (1996) was used, with the following modification: the traits respiratory noise, sudden movements of head and tearing were analyzed by percent (number of chicks by pen with presence of some of the signals mentioned before in three minutes/total number of chicks by pen, then the result was multiplied by 100).

Table 1. Control diet composition and nutrient content<sup>1</sup>

Ingredient	g/kg	Nutrient/kg	Content <sup>5</sup>
Corn grain	611.5	ME (kcal)	3035
Soybean meal	323.6	CP (g)	192.0
Soybean oil	21.4	Lysine (g)	12.7
DL-Methionine	3.3	Methionine (g)	9.3
Biolys® (51 % L-Lysine) <sup>2</sup>	4.5	Tryptophan (g)	2.5
L-Threonine	0.6	Threonine (g)	8.1
Limestone (38 % Ca)	16.3	Arginine (g)	13.1
Dicalcium phosphate (21 % Ca/18 % P)	14.7	Isoleucine (g)	10.4
Vitamin premix <sup>3</sup>	0.5	Leucine (g)	16.3
Mineral premix <sup>4</sup>	0.6	Calcium (g)	10.0
NaCl	3.0	Available phosphorus (g)	4.5

<sup>1</sup>From 1 to 28 days of age

<sup>2</sup>Also supplied per kg product: 4 g of Threonine, 1.4 g of Tryptophan, 2 g of Methionine, 1 g of Cystine, 7 g of Leucine, 6 g of Arginine, 4 g of Isoleucine, 7 g of Valine, 1.6 g of phosphorus and 4070 kcal of ME

<sup>3</sup>Supplied per kg feed: 12000 IU vitamin A; 3100 IU vitamin D<sub>3</sub>; 5 mg vitamin K<sub>3</sub>; 2 mg thiamine; 12 mg riboflavin; 21 mg pantothenic acid; 2.6 mg pyridoxine; 1.5 mg folic acid; 0.018 mg cyanocobalamin; 0.15 mg biotin. Without vitamin E

<sup>4</sup>Supplied per kg feed: 0.27 mg Se; 2 mg I; 8 mg Cu; 50 mg Fe; 80 mg Zn; 80 mg Mn; 0.2 mg Co

<sup>5</sup>Nutrient content per kg feed

## Lymphoid organs and humoral immunity

At 12, 19 and 26 days old, seven chicks per treatment were weighed, bled (by heart puncture), and then sacrificed by cervical dislocation, with the objective to collect the lymphoid organs and the serum for the immunological tests.

The lymphoid organs collected were: The bursa of Fabricius, spleen and thymus, which were individually weighed free of connective and adipose tissues adhered.

The samples of blood were allowed to clot at room temperature for one hour, and then were centrifuged at 1800 g at 4 °C for 10 minutes to obtain the serum, which was stored at -20 °C, and then were sent to the laboratory to determine the antibody titers using the hemagglutination inhibition test. Additionally, were weekly recorded the feed intake and body weight gain; also, the feed conversion ratio was calculated, from the feed intake and body weight gain data.

### Statistical analyses

Data were analyzed by a randomized complete design with equal number of replicates per treatment, using the next model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where:

$Y_{ij}$  = Response trait measured in the  $j^{\text{th}}$  replicate of the  $i^{\text{th}}$  treatment

$\mu$  = Mean that characterize the population

$T_i$  = Effect of the  $i^{\text{th}}$  treatment,  $i = 1, \dots, 4$

$E_{ij}$  = Random effect of the experimental error associated with the  $j^{\text{th}}$  replicate in the  $i^{\text{th}}$  treatment

The statistical tests were done using the GLM procedure of SAS (SAS, 2000), and means were separated using the least square means differences (LS means), contrasting each treatment against the control. The post vaccinal reaction results expressed in percent, previously to the analysis, were transformed to the arcsine of the square root of the absolute value function ( $\arcsin \sqrt{Y/100}$ ; Steel *et al.*, 1988).

## RESULTS AND DISCUSSION

### Post-vaccinal Reaction

There were differences (Table 2) in the respiratory noise percentage by the effects of the arginine and arginine plus vitamin E supplementation at 16 days old (4 days after the vaccination). When arginine (T1

vs. T2) or arginine plus vitamin E (T1 vs. T4) were supplemented in the diet, the percentage of chickens which presented respiratory noise was reduced ( $p \leq 0.05$ ). And also, the addition of 70 IU of vitamin E (T1 vs. T3) in the diet lessened the percentage of chicks with respiratory noise at 21 days old. In the tearing trait, the number of birds which presented this was lower ( $p \leq 0.05$ ) when were fed with the arginine supplemented diet (T1 vs. T2), at 21 days old. Meanwhile, in the trait sudden movements of head no differences were found in any of the sampling times.

The results found in this experiment showed the complementary role between arginine and vitamin E on the reduction of the postvaccinal reaction, because both act in different stages of this reaction. It would be because the Newcastle virus presents two stages of replication in the host; the first is during the implantation of the virus in the airways, where it replicates in the mucosal epithelium cells, passes to the blood flow and comes into the visceral organs, where it starts a second replication period, then, again is freed into the blood flow; in some cases, it can reach the central nervous system (Moreno, 1994).

These complementarities of both nutrients to improve the immune response has been reported by Abdukalykova and Ruiz-Feria (2006), who mentioned that increasing in 0.3% of arginine in the broiler chicken diets augment the antibody production four days after a sheep's red blood cells (SRBC) injection; meanwhile, the vitamin E at 80 IU remain these antibodies production at 8 and 16 days after the SRBC injection. Also, it has been reported that the concurrent supplementation of arginine (2.2 %) and vitamin E (80 IU/kg feed) regulates the cellular and humoral immune responses, due to increments in the quantities of T and B cells, and the subpopulations of lymphocytes  $CD4^+$  and  $CD8^+$  in broiler chickens vaccinated against the infectious bursitis virus (Abdukalykova *et al.*, 2008). With the results found in this experiment, it is evident that the arginine and vitamin E supplementation reduce the stress associated to the vaccination and it would benefit the health and welfare of broilers.

### Humoral immunity

Arginine at 0.3 % (T1 vs. T2) or 70 IU of vitamin E/kg of feed (T1 vs. T3) supplementation augmented ( $p \leq 0.05$ ) the antibody titers against Newcastle disease before the vaccination (11 days old); but, when they were added together (T1 vs. T4) the increment was higher ( $p \leq 0.01$ ), almost twice than the antibody titers of the birds fed the control diet (Table 3).

**Table 2.** Percentage of respiratory noise (RN), sudden movements of head (SMH) and tearing (TE)

DIET <sup>1</sup>	RN					SMH					TE					
	Age (days)															
	14	16	18	21	23	14	16	18	21	23	14	16	18	21	23	
T1	32	43	29	20	16	16	23	14	9	9	11	27	16	10	7	
T2	28	31	29	18	11	13	19	17	8	10	6	21	16	4	7	
T3	37	34	25	10	11	17	16	16	5	7	6	24	9	6	7	
T4	21	30	32	11	14	14	22	13	8	8	7	19	11	7	3	
SEM	7.7	3.7	2.5	2.9	2.6	4.6	4.2	3.5	2.3	2.5	2.5	3.8	3.5	1.6	2.2	
<b>Significance values</b>																
T1 vs. T2	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	Ns	
T1 vs. T3	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns	
T1 vs. T4	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns	

<sup>1</sup>T1 = control diet (1.31 % of arginine and 10 IU of vitamin E/ kg of feed), T2 = control + 0.3 % of arginine, T3 = control + 70 IU of vitamin E, T4 = control + 0.3 % of arginine + 70 IU of vitamin E.  
SEM= Standard error mean. \* p≤0.05; ns = not significant.

**Table 3.** Antibody titers (log<sub>2</sub>) against Newcastle disease (ND) and Avian Influenza (AI)

Diet <sup>1</sup>	ND			AI		
	Age (days)					
	11	19	26	11	19	26
T1	1.6	3.9	7.0	2.6	1.3	1.9
T2	2.7	3.0	6.1	2.1	2.0	2.0
T3	2.7	3.3	7.3	2.1	2.1	2.4
T4	3.1	3.1	6.4	2.6	2.1	2.0
SEM	0.3	0.3	0.8	0.3	0.3	0.4
<b>Significance value</b>						
T1 vs. T2	*	*	ns	Ns	ns	ns
T1 vs. T3	*	ns	ns	Ns	ns	ns
T1 vs. T4	**	ns	ns	Ns	ns	ns

<sup>1</sup>T1 = control diet (1.31 % arginine and 10 IU vitamin E/kg feed), T2 = control + 0.3 % arginine, T3= control + 70 IU vitamin E, T4= control + 0.3 % arginine + 70 IU vitamin E.  
SEM= Standard error mean; \*p≤0.05; \*\*p≤0.01; ns = not significant.

Meanwhile, at 19 and 26 days old (7 and 14 days after vaccination), the antibody titers against Newcastle disease of broiler chickens were not different ( $p>0.05$ ) in the comparisons evaluated, except for the contrast T1 vs. T2 at 19 days old. Regarding the antibody titers against avian Influenza, there were not effects of the treatments on any sampling time (Table 3).

Previous studies suggest that the arginine or vitamin E supplementation of diets for broiler chickens can increase the persistence of the maternal antibodies (Ruiz-Feria and Abdukalykova, 2009). In this trial, the basal level of antibodies against Newcastle disease augmented when the diet was supplemented with arginine or vitamin E, or both, being birds fed the last one diet which showed an antibody increment of almost twice, showing a synergism of these nutrients in the persistence of the maternal antibodies.

These results were congruent with those reported by Friedman *et al.* (1998), Leshchinsky and Klasing (2001), Abdukalykova and Ruiz-Feria (2006) and Singh *et al.* (2006); and overall make evident the modulator effect on the immune system of these two nutrients, and also, their close relationship in the metabolism and function of the immune system.

Thus, chickens fed diets supplemented with arginine, vitamin E or both, presented high levels of maternal antibodies; which indicates, that during the firsts weeks of age these birds can react better to pathogenic challenges compared to birds fed the control diet.

### Lymphoid organs weight

The weight (g/100 g of live weight) of thymus and spleen during the experiment was not affected ( $p>0.05$ ) by the dietary treatments evaluated. However, at 19 days old, the weight of the bursa of Fabricius in chickens fed arginine and vitamin E supplemented diet (T1 vs. T4) was reduced ( $p\leq 0.05$ ); but, when only arginine or vitamin E was included in the diet, there were no effects on the bursa of Fabricius weight (data not shown).

Although, there are no reports of the effects of concurrent supplementation of arginine and vitamin E on the development of the lymphoid organs, it has been demonstrated that the arginine supplementation support the development of the lymphoid organs (avoiding atrophy); however, the bursa of Fabricius is not sensitive to this amino acid concentration (Kwak *et al.*, 1999). Furthermore, the reported results about the arginine supplementation on the bursa of Fabricius weight had been controversial. For instance, meanwhile Kidd *et al.*, (2001) did not find increments

in the bursa of Fabricius weight when they augmented from 1.48 to 1.68 % the arginine levels in the diet; Tayade *et al.*, (2006) in chickens vaccinated and later challenged with the infectious bursitis virus reported that arginine at 2 % diet levels decreased the bursa of Fabricius weight from 5.43 to 2.45 g/kg of body weight.

### Performance traits

Arginine, vitamin E or their combination included in diets of broiler chickens did not show any effects ( $p>0.05$ ) on the performance traits: such as, body weight gain, feed intake and feed conversion ratio. Except at 14 days of age, birds fed arginine and vitamin E together had lower body weight (10 g approximately) in relation to those fed the control diet (T1 vs. T4,  $p\leq 0.05$ ). However, there were no other differences in the body weight in the remaining sampling times evaluated (data not shown).

Because of the immune response to the infectious processes affect the body weight gain, the metabolism and the nutrient requirements in chickens (Humphrey and Klasing, 2004), the interrelationships between nutrition and immunity are diverse and important to the animal welfare and the efficiency on performance (Humphrey *et al.*, 2002). For example, while the nutrition mediates the immunocompetence and the resistance to diseases, the pathogens can decrease the absorption of nutrients (Klasing *et al.*, 1995).

### CONCLUSIONS

The supplemented levels of arginine and vitamin E evaluated had a complementary role on the immune system. These nutrients lessened the presence of signs of the postvaccinal reaction at different stages. Moreover, it was found a synergism between these nutrients in the persistency of the maternal antibody titers against Newcastle disease virus.

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*Submitted November 10, 2011– Accepted January 12, 2012*  
*Revised received September 20, 2012*