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The Effect of High Levels of Folic Acid on Performance and Egg Quality of Laying Hens Fed on Diets with and Without Ascorbic Acid from 28-36 Weeks of Age

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Abstract: The present experiment was carried out to determine the effects of ascorbic acid and high levels folic acid added to layer hen diets on live weight gain, feed intake, feed efficiency, egg production and egg quality. A total of 270 Lohmann LSL type white layer hens at 28th weeks of age were used in the experiment. Experiment was conducted with a control and 5 experimental groups each containing 45 hens. The layers were fed basal diet, supplemented as follows; 200 mg kg^{-1} ascorbic acid (AsA), 5 mg kg^{-1} folic acid (FA1), 10 mg kg^{-1} folic acid (FA2), ascorbic acid + 5 mg kg^{-1} folic acid (AsA+FA1) and ascorbic acid+ 10 mg kg^{-1} folic acid (AsA+FA2). The groups, which had AsA+FA1 in diet, had higher body weight compared to the other groups at the end of the experiments ($p<0.01$). Feed intake was improved by addition of AsA+FA2 ($p<0.05$), but feed conversion was not effected in the group supplemented folic acid and/or ascorbic acid. Mean egg production was highest in the group including AsA and lowest in FA1 group ($p<0.01$). The average weights of eggs and egg mass were effected in the AsA+FA combination ($p<0.05$). Different levels of folic acid with or without ascorbic acid supplementation to basal diet did not produce statistically important effects on some egg quality or egg shell weight. Addition AsA+FA2 to layer diet caused a statistical increased on means shell thickness ($p<0.001$). As a result of this study, supplementation of ascorbic acid and high levels folic acid to diet positively affected live weight, feed intake and shell thickness during the early laying period.

Key words: Folic acid, ascorbic acid, performance, egg, layer

INTRODUCTION

Most layer diets today are formulated based on feed intake. With the expectation of reduced feed intake, the level of dietary nutrients such as amino acids and calcium are invariably increased (Leeson, 2007). It is unusual to adjust vitamin levels according to feed intake whereas supplementation with extra vitamins to improve the immune system or reduce the effect of stress can be considered in the context of the adverse environmental conditions present in most intensive systems (Villamide and Fraga, 1999). The performance and egg quality may be influenced by several nutrients supplemented to diet of laying hens and among these nutrients, folic acid and ascorbic acid are included. Addition of these vitamins is often recommended on the basis of stressors associated with intensive production.

The primary biochemical role of folic acid appears to be the formation of compounds such as purines, pyrimidines and certain amino acid, which involves the incorporation of a single carbon unit such as a methyl or a formyl group. It is also required for metabolism of nucleic acids and tyrosine (Lucock, 2000). No specific symptom is observed in folic acid deficiencies in poultry. However, lethargy, reduced growth rate and increased mortality rate are experienced (Maxwell *et al*., 1988). Several researchers studied the effect of high levels of folic acid supplement on egg yolk storage, performance and egg quality (Sherwood *et al*., 1993; House *et al*., 2002; Hebert *et al*., 2005). On the other hand, it is well documented that high level folic acid supplementation had no effect on overall performance, egg production and egg mass weight (House *et al*., 2002).

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Ascorbic acid is perhaps the most studied vitamin in relation to perturbation of homeostasis in poultry. Avian species have been reported to be able to synthesize ascorbic acid. However, birds are not able to synthesize sufficient ascorbic acid and increase in ascorbic acid requirements when the hens were exposed to stress caused by high air temperature or increased density birds per unit area (Njoku and Nwazota, 1989; Zakaria and Al-Anezi, 1996). It is known that ascorbic acid also has a regulatory function especially on heat stress and for steroid hormone synthesis related to heat stress. Therefore, supplemental ascorbic acid may aid overcoming any deficiency and enhance tolerance to stress (Sahin *et al*., 2003). Ascorbic acid was also reported to have a beneficial effect on egg production of laying hens and significantly decreased broken or cracked egg rate (Njoku and Nwazota, 1989; Bell and Marion, 1990).

There is interaction between folic acid and ascorbic acid. Folic acid is converted to a formyl derivative in liver. Ascorbic acid enhances the activity of liver in this reaction (Lewis *et al*., 1982). Folic acid may be involved in normal ascorbic acid metabolism because the lower ascorbic acid associated with a folate deficiency in tissue. It is also possible that the decrease in tissue ascorbic acid observed in folat deficiency is a stress response (Lewis *et al*., 1982). Folic acid and ascorbic acid have similar effects that act as antioxidant and a combination of two vitamins results in an additive effect, alleviating the negative effect of heat stress (Sahin *et al*., 2003; Gürsu *et al*., 2004).

This study was conducted to determine the effects of supplementation of higher level of folic acid on performance, certain egg quality parameters in laying hens fed with or without ascorbic acid during early laying period. Indeed, the effectiveness of supplemental dietary folic acid and ascorbic acid under practical conditions during high ambient temperature (July to August) and their interaction were investigated.

MATERIALS AND METHODS

Birds, diets and experimental design: A total of 270 Lohman LSL strain laying hens at 28 weeks old were used in this study. The experiment was conducted under appropriate animal care regulation. The birds were assigned randomly to be fed one of six isocaloric and isonitrogenous experimental diets; control diet containing neither ascorbic acid nor folic acid (C), ascorbic acid (AsA) containing diet (200 mg), 5 mg kg^{-1} folic acid containing diet (FA1), 10 mg kg^{-1} folic acid containing diet (FA2), ascorbic acid + folic acid (200 mg AsA + 5 mg kg^{-1} FA1) containing diet, ascorbic acid + folic acid (200 mg AsA + 10 mg kg^{-1} FA2) containing diet. Birds were housed in cages (50×46×46 cm). Each treatment was replicated in 9 cages with 5 hens each. The basal diet used to feed the animal in the research was formulated to be adequate in all nutrients as recommended by NRC (1994) (Table 1). During the experiment, the animals were fed ad libitum. The cages were lighted 17 h daily and the experiment lasted for eight weeks.

Table 1: Ingredients and composition of basal diet (as feed basis)

Ingredient	Content
Raw material composition (g kg^{-1})	
Corn	486.00
Wheat	88.00
Soybean Meal (47 % CP)	102.00
Full Fat Soybean	88.00
Sunflower Meal	49.00
Bone-meat meal	40.00
Poultry by-product meal	24.70
Vegetable oil	19.00
Limestone	89.00
Dicalcium phosphate	2.80
Vitamin - Mineral mixture*	7.00
Sodium chloride	2.20
DL-Methionine	1.80
Lysine	0.50
Calculated composition	
ME (MJ kg^{-1})	11.35
Dry matter (%)	90.22
Crude protein (%)	16.60
Ash (%)	13.83
Calcium (%)	4.06
Total Phosphorus (%)	0.60

*Provides per kilogram of diet; vitamin A, 28.000 IU ; vitamin D_3, 7.000 IU; vitamin E, 5.60 IU; menadione , 7 mg; tiamine, 5.6 mg; riboflavin 14 mg; vitamin B_6, 9.8 mg; vitamin B_{12}, 0.04 mg; niacin, 84 mg; D-calcium pantothenate, 22.4 mg; D-Biotin, 0.07 mg; choline chloride, 120 g; Mn, 224 mg;Fe, 112 mg; Zn, 168 mg; Cu, 14 mg; I, 5.6 mg; Co, 1.4 mg; Se, 0.42 mg

Sampling and analytical procedure: Nutritional ingredients of the experiment diets were determined according to A.O.A.C. (1984). Metabolisable energy levels were calculated using the nutritional values in the analytic results.

At the beginning and at the end of the experiment all the animals were weighed individually to determine the changes in body weight. Feed consumption was based on measurement over 15 day periods during the experimental period (60 day). Feed conversion was determined as kilograms of feed consumed per kilograms of egg produced. The total number of eggs laid per group were collected daily at the same time and recorded. The eggs collected from each group were weighed and classified as undamaged or damaged (broken, cracked and shell less).

Ninety eggs were collected randomly from the groups 2 days after every 15 days of experiment period for quality determination. Specific gravity of the collected eggs was individually measured with saline solutions. Albumen height, egg yolk diameter were measured

(Yalçin *et al*., 1990). Haug unit was calculated by using egg weight and albumen height (Nesheim *et al*., 1979). Egg shell thickness was measured with a micrometer. The measurements were taken from top, bottom and middle of the egg, the average of three values were used to obtain the egg shell thickness. The egg shell was washed, the membranes were carefully removed and left for drying for 24 h and then weighted for egg shell weights.

Statistical analysis: The data were subjected to an ANOVA with GLM model using software (SPSS, 1999). A two-way ANOVA analysis was carried out to determine the main effects (dietary folic acid and ascorbic acid concentration) and its interaction on feed intake, FCR, egg production, egg weight, albumin and yolk weight, Haugh unit and shell quality. When main effects were significant, means were separated using by the Duncan's multiple range test post hoc pair wise comparison procedure (Snedecor and Chohran, 1980).

RESULTS

Data for laying hens performance as well as external and internal indices of egg quality are presented in Table 2 and 3.

Significant differences were observed in body weight gain in response to dietary AsA+FA2 combination ($p<0.01$). The highest feed intake during the experiment was found in group FA1 with the value 95.18 g, while the lowest was found in group AsA+FA2 with the value 92.26 g. Addition of AsA+FA2 to basal diet resulted in significant ($p<0.05$) responses in feed intake. However, feed efficiency was not improved by experimental diets. During the 28-36 week period, the highest egg production (96.13%) was found in the group consuming 200 mg of AsA kg^{-1} and the lowest egg production (94.20%) was from the group receiving FA1 ($p<0.01$). Supplementation of folic acid and AsA+FA combination resulted in increased egg weight and egg mass weight ($p<0.05$). The number of damaged eggs among different treatments showed no statistical differences.

No significant difference was observed in average albumen weight, egg yolk weight, values. During the experimental period, a numerical difference in Haug unit among treatment groups was apparent although it was not statistically significant.

Two different high doses of folic acid, alone or combined with ascorbic acid, have not affected the specific gravity of the egg. Addition of ascorbic acid and/ or folic acid to basal diet had no beneficial effect on egg

Table 2:The effect of ascorbic acid and folic acid supplementation on the performance

Vitamin (mg kg^{-1} feed)		Parameters						
Ascorbic Acid (AsA)	Folic Acid(FA)	BW change, (g)	Feed intake (g)	FCR*	Egg production (%)	Egg weight(g)	Egg mass(g)	Damaged egg (%)
0	0	96.20^{b}	94.23^{b}	1.64	95.58^{ab}	62.02^{b}	57.49^{b}	0.54
	5	111.30^{b}	95.18^{b}	1.65	94.20^{b}	62.45^{ab}	57.58^{b}	0.62
	10	100.00^{b}	94.59^{b}	1.63	95.42^{ab}	62.67^{ab}	57.81^{ab}	0.93
200	0	119.10^{b}	94.51^{b}	1.62	96.13^{a}	62.21^{b}	58.31^{a}	0.69
	5	106.20^{b}	94.45^{b}	1.64	94.64^{bc}	62.81^{a}	57.54^{b}	0.70
	10	145.10^{a}	92.26^{a}	1.60	94.76^{bc}	62.93^{a}	57.60^{ab}	0.46
SEM		0.33	0.47	0.009	0.15	0.12	0.14	0.004
Source of variation				P(<F)				
AsA		0.002	NS	NS	0.01	NS	NS	NS
FA		NS[1]	NS	NS	NS	0.02	0.001	NS
AsA X FA		0.01	0.001	NS	NS	NS	0.05	NS

a-c Mean values in same column with different superscript letters were significantly different ($p<0.05$), [1]NS= Not significant ($p>0.05$), * FCR = feed conversion ratio (kg feed consumed/kg egg produced)

Table 3: The effect of ascorbic acid and folic acid supplementation on egg quality

Vitamin (mg kg^{-1} feed)		Parameters					
Ascorbic Acid (AsA)	Folic Acid (FA)	Yolk weight (g)	Albumin weight (g)	Haug unit	Specific gravity	Shell thickness (mm)	Shell weight (g)
0	0	16.76	39.95	88.86	1.0809	0.417^{c}	5.36
	5	16.49	40.49	89.64	1.0836	0.430^{ab}	5.29
	10	16.68	40.52	88.61	1.0835	0.431^{ab}	5.41
200	0	16.79	40.40	89.27	1.0835	0.429^{ab}	5.35
	5	16.97	40.54	90.17	1.0834	0.433^{ab}	5.43
	10	16.85	40.19	91.15	1.0844	0.440^{a}	5.39
SEM		0.06	0.14	0.17	0.007	0.004	0.01
Source of variation				P(<F)			
AsA		NS[1]	NS	NS	NS[1]	NS	NS
FA		NS	NS	NS	NS	0.02	NS
AsA X FA		NS	NS	NS	NS	0.02	NS

a-c Mean values in same column with different superscript letters were significantly different ($p<0.05$), [1]NS = Not significant ($p>0.05$)

shell weight. It was observed that the average egg shell thickness of the experimental groups was increased, compared to the control group depending on folic acid and ascorbic acid applications. It was found that the combination of AsA × FA2 was effective in increasing shell thickness than the other groups (($p<0.02$).

DISCUSSION

At the end of the experiment, the average body weight gain was between 96.20 and 145.10 g. It was found that AsA+FA2 combination have improved the body weight gain ($p<0.01$). Similarly, Sahin *et al.* (2003) reported that addition 1 mg of folic acid and 250 mg ascorbic acid to basal diet has beneficial effect on body weight gain in quails under heat stress.

There was a significant interaction between ascorbic acid and folic acid for feed intake ($p<0.001$). The lowest feed intake, compared to the control group, was found in AsA+FA2 group ($p<0.05$). Feed efficiency has differed between 1.60 and 1.65. A number of studies have shown that high dose folic acid supplementation to diet for laying hens has no effect on overall performance (House *et al.*, 2002; Hebert *et al.*, 2005) but, it was concluded that folic acid was shown to limited effect on feed intake (House *et al.*, 2002). Similarly, although folic acid supplementation generally improved feed efficiency in our experiment, the resulting improvement in feed efficiency was not important statistically.

In this research, birds were exposed to unfavourable environmental conditions resulting from high ambient temperature (31.4 ± 2.0°C). Sahin *et al.* (2003), reported that anti-oxidant vitamins such as folic acid and ascorbic acid needs are increased for poultry under heat stress and they effectly minimize adverse effect of heat stress on feed intake and feed efficiency of affected birds. Especially ascorbic acid requirements increase in the birds which were exposed to high temperature for a long time due mainly to they cannot synthesize enough ascorbic acid under heat stress (Cheng *et al.*, 1990; Orban *et al.*, 1993). Besides, addition of ascorbic acid to the diet had a limited effect on performance for broilers (Orban *et al.*, 1993) and commercial flocks (Creel *et al.*, 2001) during both mild heat stress and non heat stress period. This might have been because the ascorbic acid level synthesized from the body was adequate. In our study, we observed that dietary ascorbic acid and folic acid supplementation resulted in an improvement the performance (body weight gain, feed intake) compared with control group. These effects would be resulted from compensation the needs of anti-oxidant vitamins of laying hens.

Egg production of hens was different among dietary treatments (Table 2) ($p<0.05$). The only exception was egg production of birds in FA2 which was similar to control group. However, egg production of hens of FA1, AsA+FA1 and AsA+FA2 was significantly lower than the control ($p<0.05$). Eventually, the egg production was increased by the supplementation of ascorbic acid ($p<0.01$) but high level supplementation of folic acid to diet did not affect egg production. These findings are similar with other studies (House *et al.*, 2002; Çiftçi *et al.*, 2005). In contrast, according to Yiðit *et al.* (2000) who ascertained that the ascorbic acid supplement of 50, 100 and 200 mg kg^{-1} given to laying hens had no effect on egg production. On the other hand it was reported that increasing supplementation of folic acid to the level 0-64 mg kg^{-1} in the diet improved egg production (Hebert *et al.*, 2005), inadequacy of folic acid in the diet (Sherwood *et al.*, 1993) or removing vitamin B12 and choline along with folic acid from the diet decreased the egg production (Keshavarz, 2003).

Average egg weights were significantly different among treatments ($p<0.05$) AsA+F2 being highest and control group being lowest. Egg mass weights were increased by addition of folic acid ($p<0.001$) and AsA × FA interaction ($p<0.05$). This result agrees with some experiments showed that supplementation of ascorbic acid increased egg weight (Njoku and Nwazata, 1989; Orban *et al.*, 1993). There are conflicting research results concerning the effect of high levels of folic acid to the laying hen diets. House *et al.* (2002) reported that the supplementation of high level of folic acid had no effect on egg weight. Hebert *et al.* (2005) observed in a study of diet x strain interaction were observed for egg weight and the higher egg mass as a result of increased folic acid supplementations. They concluded that the increased folic acid to diets for high producing strains of birds could reduce in plasma homocysteine concentration and, the response tended to increase the production.

Some indices of internal egg quality parameters are shown in Table 3. No effect of experimental diets on albumen weight was observed. Numerical changes in the egg yolk weight were not different. Increasing of albumen and yolk weight has been found to be higher in other experiment groups compared to the control group.

It is known that weight changes of albumen and egg yolk are directly related to egg weight, the weight of the albumen is also more related to the egg weight than the weight of the egg yolk (Hussein *et al.*, 1993; Silversides and Scott, 2001). During the experiment period, changes of albumen and egg yolk weights were observed depend on the increases of the egg weight. It was also reported that the weight of the albumen increased as egg yolk weight increased and hen gets older (Hussein *et al.*, 1993). The albumen weight also increased more than the egg yolk's weight with age (O'Sullivan *et al.*, 1991).

A decrease was seen in all groups during experiment in terms of Haugh unit which is one of the inner egg quality parameters. Haugh unit is a standard method used for determining interior egg quality while being more sensitive than the usual methods. There is no detectable difference in Haugh unit with bird strain (Keener *et al.*, 2006). The reason of the decreasing of the Haugh unit is: increasing egg laying age (Akbas *et al.*, 1996; Silversides and Scott, 2001), or increasing total egg weight together with albumen weight and on the contrary decreasing albumen height (Silversides and Budgell, 2004). The decreased findings seen in our study may be due to changing albumen height and egg weight, as these were used to determine the Haugh unit.

Considering the specific gravity values about egg quality parameters of all experiment periods, the values changed between 1.0816 and 1.0840 g L^{-1} ($p>0.05$). Although, the specific gravity of egg shell is about 2.3 g L^{-1}, the density of all the eggs is a little higher than the value 1.085 g L^{-1}. For this reason, it is reported that the changes in the egg densities depend mostly on the intake of calcium (Roland, 1979) or the decreasing egg weight (Keshavarz, 2003).

In Table 3 the results of egg shell quality are shown. We observed that the shell weight numerically was generally effected by FA and AsA+ FA combination ($p<0.31$). During the complete experiment period, it was observed that the shell thickness increased with the supplementation of folic acid and interaction of AsA × FA ($p<0.02$).

The egg quality is determined by the shell quality and chemical composition criteria. Many factors such as egg size, breed, age, feeding and season affect the shell quality (Izat *et al.*, 1985). Silversides and Budgell (2004) showed that there was a correlation between total egg weight and shell weight and variation in shell weight was associated to a lesser degree with egg weight as the age of the hen confirming our results. The statistical analyses of data concerning quality of shell thickness showed difference between eggs from FA and AsA+ FA combination vs control ($p<0.02$). The supplementation of ascorbic acid to the diet has a positive effect on shell quality (Bell and Marion, 1990). The reason of this positive effect is explained as; ascorbic acid increases the calcium amount that is absorbed from intestines and transferred to the bones through the blood and affects the calcium which increases the shell structure. As a result of these, egg shell thickness and strength were claimed to be increased (Orban *et al.*, 1993). Even if the mechanism by which FA and AsA+FA combination improves eggshell thickness is unknown, it could be postulated that folic acid and ascorbic acid are two anti-oxidant vitamins (Sheehy *et al.*, 1997; Joshi *et al.*, 2001), they might be effected on the retention of nitrogen and minerals likely are attributable to the protection of the pancreas from oxidative stress (Sahin *et al.*, 2003). These beneficial protective effects of vitamins may contribute to egg formation.

We observed that slightly positive effect of supplementation with ascorbic acid alone on the shell thickness. The lack of response would be resulted from an insufficient dosage of ascorbic acid unable to totally recover ascorbate requirement. Conversely, the concentration of tissue ascorbic acid is differed by strain of bird and the weak eggshell production is not associated with reduced ascorbic acid synthesis (Maurice *et al.*, 2004).

Although, there were statistical significant effects of AsA+FA combination on shell thickness observed over entire feeding period but the damaged eggs rate was not significantly affected by the vitamins supplementation, although lower rate was observed in AsA+FA2 group in comparison with control (Table 2). Our results showed that folic acid and/or ascorbic acid may be ineffective on the damaged egg rate in hens during early laying period (28-36 week). The present results are agreement with some reports that the eggshell percentage and amount of shell per surface were higher in layers with early laying period (30 week of age) compared with late period (52 week of age) (Panheleux *et al.*, 2000) and after 30 week of age, birds had the best shell quality as seen lower deformation (Leeson and Caston, 1997).

CONCLUSION

In the present study, although the basal diet contained enough folic acid, a level of folic acid was well below the calculated level of folate in unsupplemented diet (0.37 mg kg^{-1}) to the recommended level of 0.21-0.31 mg kg^{-1} (NRC, 1994), the high level supplementation of folic acid (5 and 10 mg kg^{-1}) with and without ascorbic acid (200 mg kg^{-1}) to diet improved performance. In conclusion, supplementing high levels of folic acid and ascorbic acid and their combinations to the diet has positive effects for the peak production level laying hens. These positive effects are live weight, feed intake, egg weight and egg mass together with egg shell quality. These results indicated that feeding layer with a diet containing 5 mg, 10 mg folic acid/kg diet and/or ascorbic acid (200 mg kg^{-1}) have some beneficial effects in practice. The use of diets with high level folic acid and ascorbic acid during peak production period may overcome the practical difficulties.

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