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Effects of Ascorbic Acid on Egg Production and Egg Shell Quality in Laying Hens Drinking Saline Water

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Abstract: An experiment was conducted to study the effects of different water source, saline water (Tap water +2 g L^{-1} Na) and tape water with a diet that supplemented by three level of Ascorbic Acid (0, 1.5 and 3 g kg^{-1} of diet) on laying hen's performance and egg shell quality. This trail was carried out on 108 commercial laying hens (Hy-Line) 32 weeks old for 12 weeks. All of the data were subjected to Two- way analysis of variance test. The result showed that egg production (%), egg weight (g), egg mass (g/h/day), feed conversion ratio, feed consumptions didn't effected by saline water but using saline water increased the percentage of eggs with damaged shells ($p<0.01$). Also the effect of different level of Ascorbic acid on egg production (%), egg weight (g), egg mass (g/h/day), feed conversion ratio were not significant, but feed consumption reduced by using ascorbic acid ($p<0.05$). Egg shell thickness (mm), egg shell weight (%), egg shell weight (mg/cm^2), egg specific gravity, egg shell calcium (%) and egg shell phosphorous (%) didn't affected by saline water and different level of Ascorbic acid.

Key words: Water sources, ascorbic acid, egg production, shell quality

INTRODUCTION

Underground water supplies are a common source of drinking water for poultry in many countries and such water often contains high concentrations of dissolved mineral salts. These salts can contribute to the mineral requirements of poultry although their presence is usually not considered when estimates of requirements are made (Ross, 1979). However, when present in high concentrations many minerals can prove toxic and it is under these conditions that reductions in growth and laying performance can occur. Sodium and Chloride ions contribute to the production of defective shells in eggs from hens receiving NaCl supplements in the drinking water (Yoselewitz *et al*., 1988; Balnave, 1996).

As we know underground water is one of the most important sources of drinking water for poultry in Iran and such water often contains high concentrations of NaCl in many provinces. The level of salt in underground water is dependent to level of raining, thus its can changed during the years and because of saline water the percentage of egg with damaged eggshells is high. Unfortunately this problem didn't noticed by producer and it's make an economical problem for poultry industry.

Several studies were conducted to improve the negative saline water effects. For example many studies showed that using zinc soleplate in layers diet improved egg shell quality by increasing the anhydric carbonate activity (Balnave and Zhang, 1992). Scientist try to solve the problem by supplementation of saline water with ammonium bicarbonate (Yoselewitz and Balanave, 1990), ascorbic acid (Balnave *et al*., 1991; Balnave and Zhang, 1992; Kolapo *et al*., 2002), zinc- methionine (Morenge *et al*., 1992) and zinc sulphate (Morenge *et al*., 1992). Ascorbic acid is a vitamin reported to have beneficial effects in poultry exposed to environmental or nutritional stress (Thornton and Moreng, 1959; Pardue and Thaxton, 1986; Krautmann, 1988). Egg shell quality is one factor showing improvement, especially at high ambient temperatures, although the response in individual experiments have been inconsistent (Pardue and Thaxton, 1986).

According to the problem and beneficial effect of vitamin C on egg shell, in these research we try to study about supplementation of ascorbic acid to laying hen's diet in order to decreasing the negative effects of saline drinking water.

MATERIALS AND METHODS

According to industry problem about saline water effects on eggshell quality in Iran the effects of Ascorbic Acid on egg production and egg shell quality in laying hens drinking saline water was investigated in Department

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Table 1: The level of minerals and other quality of examined water

pH	7.9
EC (Electrical conductivity) (Umhos Cm^{-1})	940
TDS (mg L^{-1})	636
Na^{+} (meq L^{-1})	6.6
Ca^{2+}+Mg^{2+} (meq L^{-1})	4.4
Cl^{-} (meq L^{-1})	2.4
So_4^{2-} (meq L^{-1})	3.6
HCO_3^{-} (meq L^{-1})	4.7

Table 2: Composition of diets in different groups

Ascorbic acid levels (g kg^{-1} diets)			
ingredient (%)	0	1.5	3
Corn	58.55	58.56	58.58
Soybean meal	11.41	11.43	11.44
Barely	10	10	10
Fish meal	5	5	5
Salt	0.17	0.18	0.18
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL- methionine	0.03	0.03	0.03
Wheat bran	3.37	3.32	3.26
L- lysine	-	-	-
Fat (plant)	3	3	3
Oyster shell	7.88	7.88	7.88
Nutrient			
Fat (powder)	-	-	-
ME (kcal kg^{-1})	2900	2900	2900
Crude protein (%)	14.85	14.85	14.85
Crude fiber (%)	3.08	3.08	3.08
Methionine (%)	0.33	0.33	0.33
Methionine +cysteine (%)	0.58	0.58	0.58
Lysine (%)	0.79	0.79	0.79
Argenine (%)	0.86	0.86	0.86
Calcium (%)	3.25	3.25	3.25
Available phosphorous (%)	0.25	0.25	0.25
Na (%)	0.15	0.15	0.15
Cl (%)	0.16	0.16	0.16
K (%)	0.53	0.53	0.53
Zn (%)	0.011	0.022	0.033
Anion-cation balance (meq/100 g diet)	15.51	15.51	15.51

[1]Vitamin premix supplied per kg of diet: Vitamin A, 8000 IU; Vitamin D3, 3500 IU, Vitamin E, 70 IU, Vitamin K, 5 mg, Vitamin C, 300 mg, cholin chloride, 1000 mg, [2]Mineral premix supplied per kg of diet: Fe, 250 mg; Cu, 100 mg; Zn, 150 mg; Se, 15 mg and Mn 100 mg

of Animal and Poultry Nutrition of Animal Science Research Institute in Karaj, Iran. This study was conducted with white Leghorn hens (Hy-Line W36), aged 32 to 44 weeks. At the age of 20 weeks the natural day length was artificially increased from 11 h/day to 16 in peak of egg production and then it maintained constant until the end of experiment.

One hundred and eight hens at 32 week of age were selected according to egg production and weight and then they were randomized into individual layer cages. Hens were kept in confinement housing under semi controlled environmental conditions. The experiment consisted of 2 source of water (town water and saline water) and 3 levels of Ascorbic Acid in diets (0, 1.5 and 3 g kg^{-1} of diet) with three replicates each containing 6 laying hens. In this periods birds fed with 3 diets (Table 2). Water minerals (Ca, Na, Mg, Cl, SO_4, Co_3) and pH, TDS were measured (Table 1) according to Alison *et al.* (1953) methods.

In experimental period egg production (%), egg weight (g), mortality (%) and feed intake (g) were measured weekly and egg specific gravity, egg shell thickness (mm), egg shell weight (mg cm^{-2}), egg shell weight(g), egg mass (g/h/day) and percent of calcium and phosphorous in egg shell were investigated.

Statistical analysis: All of the data were subjected to Two-way analysis of variance test. Statistical significances among treatment means were determined by the method of new multiple range test of Duncan (1955) when the F-value was significant at 5% level.

RESULTS AND DISCUSSION

Result

Feed intake: The result (Table 3) showed that type of water had no effect on feed intake, but using ascorbic acid reduce the feed intake ($p<0.05$). The comparison of treatment showed that feed intake for zero level in tape water and saline water source was higher than other groups ($p<0.05$).

Egg production: Egg production didn't effected by using kind of water source and using of ascorbic acid. The difference of treatment wasn't significant ($p<0.05$). But the percentage of eggs with perfect egg shell was reduced by using saline water. The comparison treatment showed that egg production in 0 groups was 4.7% higher than of ascorbic acid groups but the differences weren't significant. Using Ascorbic acid can't reduce the percentage of egg with damaged shell when birds used tape water but in saline water the percentage of egg with damaged shell (31.95%) improved by using 3 g ascorbic acid.

Egg weight: There was no significant difference between tape and saline water effect on egg weight (g) and also the effect of ascorbic acid was not significant. But the comparison of treatment showed that in saline water the group of 0 and 3 (g ascorbic acid/kg of diet) had a best egg weight ($p<0.05$).

Egg mass: Egg mass (g/h/day) didn't affected by kind of water and using different level of Ascorbic acid. The comparison of data showed that there was no difference between treatments, but egg mass for Zero groups in both water source and 3 g Ascorbic acid per kg of diet was the best.

Table 3: The effect of tape, saline water and ascorbic acid on layer performance

Water source	Ascorbic acid level (g kg^{-1})	Egg production (%)	Egg with perfect shell (%)	Egg with damaged shell (%)	Egg weight (g)	Egg mass (g/h/day)	Feed intake (g/day/h)	Feed conversion	Body Weight gain (g)
	0	89.08±0.4	87.36±5.4^{a}	12.64±5.4^{b}	57.90±0.6^{ab}	51.60±0.7	109.6±1.5^{ab}	2.12±0.06	55.88±27.1
Tape water	1.5	85.02±4.4	87.78±4.62^{a}	12.22±4.6^{b}	58.40±1.3^{ab}	49.68±2.6	108.6±6.1^{b}	2.19±0.16	87.11±48.8
	3	84.57±4.1	85.76±1.01^{a}	14.24±1.0^{b}	56.60±.78^{b}	47.99±2.6	109.1±1.6^{b}	2.27±0.14	124.00±38.1
Saline water	0	89.15±3.0	74.43±3.36^{b}	25.57±3.4^{a}	58.80±1.9^{a}	52.47±3.4	112.9±3.9^{a}	2.15±0.09	103.78±3 8.3
	1.5	85.24±2.0	73.54±6.0^{b}	26.46±6.0^{a}	57.18±1.2^{ab}	48.74±1.4	107.7±3.9^{b}	2.21±0.06	52.53±20.1
	3	85.97±1.3	84.6±2.5^{a}	17.40±2.5^{b}	58.80±1.5^{a}	50.58±1.7	112.3±2.3^{a}	2.20±0.06	127/77±6
Water		NS	**	**	NS	NS	NS	NS	NS
Ascorbic Acid	NS	NS	NS	NS	NS	*	NS	NS	
Interaction		NS	*	*	NS	NS	NS	NS	NS

NS = None Significant, * Significant difference ($p<0.05$) ** Significant difference ($p<0.01$), ^{A,b} Mean within a column followed by different superscripts statistically different ($p \leq 0.05$)

Table 4: The effect of tape, saline water and ascorbic acid on layer hen eggs quality

Water source	Ascorbic acid level (g kg^{-1})	Egg with cracked shell (%)	Egg without shell (%)	Egg shell thickness (mm)	Egg shell weight (%)	Egg shell weight (mlg cm$^{-2)}$	Egg specific gravity	Egg shell Calcium (%)	Egg shell Phosphorous (%)
	0	11.30±5.4^{b}	0.073±0.13	0.360±0.02	8.77±0.46	72.30±3.2	1.079±0.0	37.24±6.4	0.068± 0.0
Tape water	1.5	9.830±3.49^{b}	0.273±0.41	0.366±0.01	8.84±0.24	74.00±1.2	1.078±0.0	39.40±3.5	0.070±0.01
	3	11.56±1.24^{b}	0.000±0.00	0.349±0.01	8.62±0.15	71.57±1.4	1.077±0.0	37.40±6.8	0.068±0.0
	0	22.11±5.32^{a}	0.380±0.34	0.353±0.0	8.79±0.2	73.53±1.3	1.078±0.0	37.82±6.3	0.070±0.00
Saline water	1.5	23.42±5.9^{a}	0.460±0.40	0.346±0.02	8.71±0.65	72.11±5.1	1.078±0.0	38.44±3.6	0.067±0.00
	3	14.99±1.25^{b}	0.857±1.09	0.356±0.01	8.66±0.20	72.14±2.1	1.078±0.0	36.97±7.5	0.073±0.01
water		**	NS	NS	NS	NS	NS	NS	NS
Ascorbic Acid		NS	NS	NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS	NS	NS	NS

NS = None Significant ** = Significant difference ($p<0.01$), ^{a,b} Mean within a column followed by different superscripts statistically different ($p \leq 0.05$)

Feed conversion ratio: Different source of water and level of ascorbic acid had no positive effect on feed conversion ratio and also there was no difference between treatments. But zero groups in both water source had a best feed conversion ratio.

Egg shell quality: The percentage of egg without shell, egg shell thickness (mm), egg shell weight (%), Egg shell weight (mg cm^{-2}), egg specific gravity, egg shell Calcium (%) and egg shell phosphorous (%) didn't effected by different kind of water. Also effect of different level of ascorbic acid was not significant.

The comparison of treatment showed that using 3 g Ascorbic acid per kg of diet can reduce the egg with damaged eggshell (approximately 32.2%). Using 3 g Ascorbic acid per kg of diet in tape water decreased the percentage of egg without shell (Up to Zero) (Table 4).

DISCUSSION

The production of defective shells in eggs was similar to Yoselewitz *et al.* (1988) that they reported sodium and chloride ions contribute to the production of defective shells in eggs from hens receiving NaCl supplements in the drinking water. The primary metabolic lesion associated with the poor eggshell quality which results from the intake of saline drinking water appears to be related to the supply of bicarbonate rather than calcium to the lumen of the shell gland for eggshell formation. A reduced activity of carbonic anhydrase in the shell gland mucosa is the particular importance. This limits the supply of bicarbonate and the dependent calcium to the lumen of the shell gland. Using 3 g ascorbic acid per kg of diet can reduce the egg with damaged eggshell (approximately 32.2% in this experiments) but the mechanism by which ascorbic acid exerts its effect is unknown. Vitamin C has a several roles, for example reducing the effects of stress, ant oxidation effects and improving immune response that these roles maybe help to improve the saline water effects.

In this experiment saline water had no negative effect on egg production that it maybe because of the amount of salt in water because as we noticed Krista *et al.* (1961) showed that 7 g L^{-1} had no effect. Krista *et al.* (1961) reported that when given over a 16-week period, 10 g NaCl L^{-1} of drinking water significantly decreased egg production in laying hens, although 7 g L^{-1} had no effect.

In this experiment the percentage of calcium and phosphorus of eggshell didn't affected by using saline water, but Pourreza *et al.* (1994) reported that shell calcium and shell thickness were reduced. This variation is maybe because of the level of salt that received by birds, in this experiment the level of salt in diet is normal and the birds are exposed only by saline water. When the level of salt consumption increased, excess consumption of chloride

causes metabolic acidosis (Keshavarz and Austic, 1990) and lead to a reduction in blood bicarbonate. Reduced blood bicarbonate results in reduced bicarbonate concentrations in uterine fluid and impaired eggshell formation (Balnave *et al*., 1989). Reduction in the bicarbonate concentration in the uterus impairs shell formation and thereby decreases utilization of blood calcium, leading to reduced shell quality.

Using 3 g ascorbic acid per kg of diet improved the percentage of egg with perfect shell. This result was disagree with Kolapo *et al*., (2002) reports, they reported that there were no effects of source of water (tape and rain water), or level of ascorbic acid supplementation, on egg production traits and egg quality. The result of this experiment was agree with Balnave and Zhang (1992) result that the adverse effects of drinking water containing salt at 2 g L^{-1} were prevented by ascorbic acid supplementation at the rate of 800 ppm. The lack of ascorbic acid supplementation on egg weight contrasts with the findings of Perek and Kendler (1962) reported that supplementation with this vitamin led to production of heavier eggs. Presumably there were adequate amounts of this vitamin in the basal diet. This contrast was maybe because of level of Ascorbic acid in diet.

Under the condition of this study, using 3 g Ascorbic acid per kg of diet can improved the negative effects of saline water (2 g L^{-1}) on egg shell quality and Ascorbic acid especially improved the percentage of egg with perfect shell.

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