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■Author(s)

Moravej H Alahyari SM* Shivazad M

Department of Animal, Faculty School of Agriculture and Natural Resources, The University of Tehran, Karaj, 31587-77871, Iran

■Mail Adress

Corresponding author Tel: +989351103551; fax: +982612249752; E-mail: Majid.alahyari@ut.ac.ir

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Submitted: December/2012 Approved: August/2012 *Effects of the Reduction or Withdrawal of the Vitamin Premix from the Diet on Chicken Performance and Meat Quality*

ABSTRACT

The present study was carried out to examine the effect of vitamin premix reduction or withdrawal in finisher diet (29 to 42 days of age) on the performance and meat quality of chicks fed a wheat/barley based diet and reared on floor pens. A total of 504 male broiler chicks (Ross® 308) were allocated to seven treatment groups, with four replicates per treatment and 18 birds per replicate. All data were analyzed according to a completely randomized design. Results showed that the reduction or withdrawal of the vitamin premix from diets in different weeks of finisher period did not affect chicken performance (p>0.05). There were no significant differences in TBARS values of thigh samples of birds slaughtered at 35 days of age. However, the TBARS value of the treatment without vitamin premix was significantly higher than in the other treatments (p < 0.05) when birds were slaughtered at 42 days of age. Finally, the results of this study demonstrated that it is not possible withdrawal all premix vitamin of the diet. However, it may be possible to reduce vitamin supplements in finisher broiler diets without any negative effects on performance or meat guality during freezing.

INTRODUCTION

The removal of vitamin supplements from broiler finisher diets has received considerable attention during the past few years. Vitamins are organic compounds present in most feedstuffs in minute amounts. However, vitamins are essential for normal metabolism. Also, they can cause specific deficiency diseases if absent from the diet and cannot be synthesized by the host animal to meet vitamin requirements (Coelho & McNaughton, 1995). Several reports have demonstrated that vitamin premix removal from broiler finisher diets based on corn did not significantly affect broiler performance or humoral immune response (Skinner *et al.*, 1992; Christmas *et al.*, 1995; Maiorka *et al.*, 2002; Khajali *et al.*, 2006). Further studies on the withdrawal or reduction of vitamin supplements in finisher diets are required because:

1. It is not clear that the level of vitamin E in broilers meat is sufficient to stabilize quality after slaughter and long storage times in the freezer. Lipid oxidation is the main cause of meat quality deterioration, because products of autoxidation of unsaturated fatty acids affect the wholesomeness and nutritional value of the final product (Pearson *et al.*, 1993). Lipid oxidation is an important determinant of shelf life of meats and meat products. Lipids in poultry show higher degree of unsaturation than red meats due to a relatively high content of phospholipids (Igene & Pearson, 1979). The degree of unsaturation of the phospholipids of the subcellular membrane is an important factor in determining the oxidative stability of meats, with the oxidative potential



increasing as the degree of unsaturation of meat lipids increases. Phospholipids are located in membrane structures, and the initiation of lipid peroxidation in tissues has been tracked to the membrane level (Machlin, 1984). Vitamin E is part of the body's intracellular defense against the adverse effects of reactive oxygen species and free radicals that initiate oxidation of unsaturated phospholipids (Chow, 1979).

2. There is a lack of reports on the removal vitamin premix from broiler finisher diets based on wheat and barley. The volatility of global corn prices may the use of wheat and barley instead of corn in broiler diets. Also, there are differences between vitamin content in wheat, barley and corn.

Therefore this study was carried out to evaluate the effects of the reduction or withdrawal of vitamin supplements from diets based on wheat and barley during the finisher period on performance and meat quality of broilers.

MATERIAL AND METHODS

General Proe

Birds and housing

Chick average initial body weight was 42 g. Room temperature was kept at 34°C during the first three days of the trial and then gradually reduced as bird aged until 22°C at 21 days of age. Continuous light was supplied during the first three days, after which the lighting regimen was 23 hours/day. Chicks were raised up to 29 days of age and were fed commercial starter and grower diets that met their nutritinal requirements (Aviagen, 2007). Male broiler chicks (Ross 308) were distributed into pens according to completely randomized design with seven treatments with four replicates of 18 birds per replicate. Birds were reared in floor pens.

Dietary treatments

Treatments are described in Table 1: T1 – feed with no vitamin premix (VP), from 29 to 42 days of age; T2 – 33% VP, from 29 to 42 days of age; T3 – 33% VP from 29 to 35 days of age and no VP from 36 to 42 days of age; T4 - 66% VP, from 29 to 42 days of age; T5 – 66% VP from 29 to 35 days of age and no VP from 36 to 42 days of age; T6 – 100% VP, from 29 to 42 days of age; T7 – 100% VP from 29 to 35 days of age and no VP from 36 to 42 days of age.

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The ingredient and nutritional composition of the experimental diets are shown in Table 2. Mash feed and water were offered *ad libitum*.

Table	1	-	Composition	of	diets	used	during	the
experin	ner	ntal	period (29 thr	ougł	142 d	of age)		

Ingredients (%)	Treat- ment1 ¹	Treat- ment2/3	Treat- ment4/5	Treat- ment6/7		
Wheat	36.38	36.05	35.97	35.79		
Barley	30.00	30.00	30.00	30.00		
Soybean meal (44%)	27.93	28.09	28.04	28.09		
Soybean oil	2.74	2.80	2.86	2.90		
Oyster shell	1.24	1.25	1.24	1.24		
Dicalcium phosphate	0.89	0.90	0.90	0.90		
Vitamin premix ²	0.00	0.08	0.16	0.25		
Trace mineral premix ³	0.25	0.25	0.25	0.25		
Sodium chloride	0.28	0.28	0.28	0.28		
DL-Methionine	0.17	0.18	0.17	0.17		
L-Lysine-HCI	0.07	0.07	0.07	0.07		
Multi Enzyme (Rova- bio®)4	0.05	0.05	0.05	0.05		
Total	100	100	100	100		
Calculated composition#				·		
ME (kcal/kg)	2,900	2,900	2,900	2,900		
CP (%)	20.00	20.00	20.00	20.00		
Met (%)	0.37	0.37	0.37	0.37		
Met + Cys (%)	0.73	0.73	0.73	0.73		
Lys (%)	0.97	0.97	0.97	0.97		
Na (%)	0.16	0.16	0.16	0.16		
Ca (%)	0.76	0.76	0.76	0.76		
Available P (%)	0.37	0.37	0.37	0.37		

 1 T1 – Without VP, during 29 to 42 days of age; T2 – 33% VP, during 29 to 42 days of age; T3 – 33% VP during 29 to 35 days of age and without VP during 36 to 42 days of age; T4 - 66% VP, during 29 to 42 days of age; T5 – 66% VP during 29 to 35 days of age and without VP during 36 to 42 days of age; T6 – 100% VP, during 29 to 42 days of age; T7 – 100% VP during 29 to 35 days of age and without VP during 36 to 42 days of age and without VP during 36 to 42 days of age; T7 – 100% VP during 29 to 35 days of age and without VP during 36 to 42 days of age.

² 2.5 kg of vitamin premix contained: 2700mg retinal, 400mg calcidiol, 18g tocopheryl acetate, 2000mg menadione, 1800mg thiamine, 6600mg riboflavin, 10g niacin, 30g calcium pantothenate, 3g pyridoxine, 1g folic acid, 15mg cobalamin, 250g choline chloride, 100mg biotin.

 $^{\rm 3}$ 2.5 kg of trace mineral premix contained: 100g Mn, 50g Fe, 100g Zn, 10g Cu, 1g I, 200mg Se.

⁴ This product contained mainly β -glucanase and xylanase activities. The endo-1,3(4)- β -glucanase 100 AGL/kg diet and endo-1,4- β - xylanase 70 AXC units/ kg diet. #Based on a dry matter content of 86%.



Table 2 – Composition of the starter and grower diets used in the pre-experimental phases.

Ingredients (%)	Starter diet	Grower diet		
Wheat	34.00	35.14		
Barley	32.00	30.00		
Soybean meal (44%)	23.97	26.93		
Corn Gluten meal	5.62	2.51		
Soybean oil	1.03	1.80		
Oyster shell	1.30	1.29		
Dicalcium phosphate	1.05	1.05		
Vitamin premix ¹	0.25	0.25		
Trace mineral premix ²	0.25	0.25		
Sodium chloride	0.28	0.28		
DL-Methionine	0.07	0.21		
L-Lysine-HCl	0.13	0.24		
Multi Enzyme (Rovabio®) Total	0.05 0.05 100 100			
Calculated composition				
ME (kcal/kg)	2,850	2,870		
CP (%)	20.80	20.00		
Met (%)	0.48	0.41		
Met + Cys (%)	1.00	0.86		
Lys (%)	1.35	1.12		
Na (%)	0.15	0.14		
Ca (%)	0.99	0.81		
Available Phosphorus (%)	0.47 0.41			

¹ 2.5 kg of vitamin premix contained: 2700mg retinal, 400mg calcidiol, 18g tocopheryl acetate, 2000mg menadione, 1800mg thiamine, 6600mg riboflavin, 10g niacin, 30g calcium pantothenate, 3g pyridoxine, 1g folic acid, 15mg cobalamin, 250g choline chloride, 100mg biotin.

 $^{\rm 2}$ 2.5 kg of trace mineral premix contained: 100g Mn, 50g Fe, 100g Zn, 10g Cu, 1g I, 200mg Se.

Prior to formulation, all main dietary ingredients were analyzed for AMEn, amino acid (AA) profile (according to prediction formula pulbished in NRC, 1994), crude protein (CP), crude fiber (CF), and ether extract (EE) contents, as described by AOAC (2000).

Productive performance traits

At 29 days of age, body weights were similar in all pens (1135±14 g). Mortality from 29 to 42 days was determined for each pen. Feed intake and body weight were determined at 29, 35 and 42 days of age. Feed conversion ratio was based these data and calculated per period and for the complete experimental period. Feed intake was adjusted for mortality. Birds that died during the experiment were weighed, and their body weight was included in calculations of feed conversion

ratio. Feed waste was recorded per replicate for each period.

Thiobarbituric acid (TBARS) value

Thighs from two birds slaughtered per replicate were stored in individual oxygen-permeable plastic bags at -20°C (frozen storage) until chemical analysis performed after 180 days. Thighs were thawed and ground, and their oxidative stability was measured in TBARS values. The TBARS value (expressed as malonaldehyde equivalents) was colorimetrically determined by the method of Porkony & Dieffenbacher (1989), as described by Kirk & Sawyer (1991). A portion (200 mg) of sample was weighed into a 25mL volumetric flask. An aliquot (1 mL) of 1-Butanol was added to dissolve the sample. The mixture was made to volume and mixed. A portion (5 mL) of the mixture was pipetted into a dry stopper test tube and 5 mL of TBA reagent (prepared by dissolving 200 mg of 2-TBA in 100 mL 1-butanol, filtered, stored at 4°C for not more than seven days) were added. The test tubes were closed, vortexed and placed in water bath at 95°C for 120 min and then cooled. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) was recorded. TBA Value (mg of malonaldehyde/kg of tissue) was obtained by the formula:

$$TBARS = \frac{(A - B) \times 50}{M} \times 1000$$

Where:

A is the absorbance of the test solution

B is the absorbance of the reagent blank

M is the mass in mg, of the test portion

50 is a factor valid if the volume of the volumetric flask is 25ml and cell width 10mm (Mihara & Uchiyama, 1978).

Statistical Analysis

Data were submitted to analysis of variance (ANOVA) as a completely randomized design using the GLM procedures of SAS software package (SAS, 2002). Means were compared by Duncan's Multiple Rang Test at a significance level of p<0.05.

RESULTS

Mortality for all groups was within the expected range and was not significantly different among treatments. Average daily feed intake (ADFI), body



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	29-35d			36-42d			29-42d			
Treatments	Average daily feed intake (g/day)	Body weight gain (g/day)	FCR ¹	Average daily feed intake (g/day)	Body weight gain (g/day)	FCR	Average daily feed intake (g/day)	Body weight gain (g/day)	FCR	Body weight 42 day (g)
T 1	145	85	1.71	172	91	1.88	159	88	1.80	2366
Т 2	143	83	1.73	169	89	1.91	156	86	1.82	2361
Т 3	145	87	1.66	169	86	1.95	157	87	1.81	2390
Т4	146	86	1.70	169	96	1.80	157	91	1.73	2395
Τ5	147	86	1.70	170	94	1.81	158	90	1.76	2393
Т б	145	87	1.66	172	94	1.84	159	90	1.75	2395
Τ7	146	87	1.68	171	93	1.85	159	90	1.77	2391
SEM	3.4	17.52	0.05	4.1	5.1	0.07	2.6	3.0	0.04	25.4

¹ Feed conversion ratio (FCR) (g/g)

weight gain (BWG), and feed conversion ratio (FCR) results are shown in Table 3. VP reduction or withdrawal at different ages did not significantly affect ADFI, BWG or FCR (p > 0.05).

The TBARS values of thigh samples after six months of storage in a in freezer are presented in Table 4. TBARS values of the thigh samples of birds slaughtered at 35 days of age were not significantly different (p > 0.05). However, TBARS values of T1 chicks were significantly higher than relative to the other treatments (p < 0.05), when birds were slaughtered at 42 days of age.

Table 4 – Effect of storage time on the oxidative stability (TBARS values – mg malondialdehyde/kg tissue) of thighs from birds were slaughtered at 35 and 42 days of age.

T 1	TBA 35 d	TBA 42 d
	1.66	3.57 ^b
T 2	1.36	2.18ª
Т 3	1.53	1.83ª
T 4	1.27	1.83ª
T 5	1.49	1.85ª
Τ 6	1.37	1.81ª
Τ7	1.35	1.88ª
SEM	0.15	0.16

 $^{\rm ab}$ Values in the same column within each experiment with different superscripts differ significantly (p < 0.05).

DISCUSSION

Performance

Although vitamins do not supply energy, they act as cofactors in several metabolic reactions, thus increasing the efficiency of synthesis pathways in the animal. Because broilers have high vitamin requirements during rearing, it would be expected that reducing dietary vitamin levels during the finisher period would increase feed conversion.

The findings of the present study were consistent with those reported by Skinner *et al.* (1992) and Khajali *et al.* (2006), who showed that vitamin and mineral premix withdrawal from broiler finisher diets did not affect their performance. Skinner *et al.* (1992) suggested that the lack of a withdrawal effect could be related to the availability of vitamins and minerals in the body for further growth, as the amount of these supplements usually exceeds two or three times the recommended broiler requirements.

On the other hand, omitting vitamins from the finisher diet for the same period decreased weight gain in three different broiler strains (Deyhim & Teeter, 1993; Patel *et al.*, 1997). Deyhim & Teeter (1993) also demonstrated that broilers reared in battery cages, under cycling ambient temperature (24 to 35°C, creating heat stress), and fed diets without vitamin and mineral supplement presented reduced weight gain, increased mortality and compromised feed conversion when compared with birds fed normally supplemented diets. These differences may be due to the type of rearing system (floor or cages) or differences in diet composition.



Lipid Oxidation in Thigh Meat

Vitamin E functions as a guenching agent of freeradical molecules presenting single, highly reactive electrons. Highly reactive oxygen species, such as superoxide anion radical (O₂), hydroxyl radical (HO), hydrogen peroxide (H_2O_2) , and singlet oxygen (O_2) , are continuously produced in the course of normal aerobic cellular metabolism. Also, phagocytic granulocytes undergo respiratory burst to produce oxygen radicals to destroy intracellular pathogens. However, these oxidative products can, in turn, damage healthy cells if they are not eliminated. Antioxidants serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells (Chew, 1995; McDowell, 2000). Therefore, antioxidants are very important to animal health. Vitamin E oxidation prevents the oxidation of lipids and consequent formation of free radicals and peroxides within cells, protecting the cell membrane from damage (Drouchner, 1976). If lipid hydroperoxides are allowed to form in the absence of adequate tocopherols, direct cellular tissue damage can result, in which peroxidation of lipids destroys structural integrity of the cell and causes metabolic disorders. Vitamin E reacts or functions as a chain-breaking antioxidant, thereby neutralizing free radicals and preventing oxidation of lipids within membranes. Free radicals may not only damage their cell of origin, but migrate and damage adjacent cells in which more free radicals are produced in a chain reaction leading to tissue destruction (Nockels et al., 1996). Bartov & Frigg (1992) showed that oxidative stability was best in tissues from broilers that received vitamin E continuously during the trial and presumably had higher alpha-tocopherol contents in the meat. However, it seems that withdrawal of vitamin premix did not have significantly effects on broiler performance, but it can damage tissues by decreasing the vitamin E requirements for stable quality meat during freezing.

CONCLUSIONS

The results of the present study demonstrated that it is not possible to apply full vitamin premix withdrawal in finisher broiler diets; its levels may be reduced with no negative effects on performance or meat quality during freezing.

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