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Comparison of Different Levels of Vitamin Premix on Meat Lipid Oxidation in Floor and Battery Cage Broiler Raising Systems

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Abstract

A total of 1260 male broiler chicks (Ross 308) were allocated to 7 treatment groups (different levels and access time to vitamin premix (VP)) at 29 to 42 days of age, in floor system (Experiment 1) and battery cage system (Experiment 2). The oxidative stability was evaluated by thiobarbituric acid reactive substances (TBARS) on the thigh yield that were storage for 180 days in -20˚C. Results of experiment 1 showed that VP reduction and withdrawal from 29 d of age did not impair body weight, feed intake and feed conversion ratio during the final period of treatments (29-42d).Results of experiment 2 showed that there were no significant differences in the birds' performance with reduction or withdrawal of VP from diets in 29-35 days among the experimental groups, but in 36-42 days of age, the performance of the group of birds fed with diet without VP (T1) was significantly lower than other groups. The results of experiment 2 showed that the performance of birds fed with diet without VP was significantly lower than other treatments in 36-42 days of age. The results of TBARS values in experiment 1 for thigh meat samples of birds slaughtered at 42 days of age showed that TBARS values of treatments without VP were significantly higher than other treatments. However in experiment 2, TBARS values of treatments without VP and with 33% VP were significantly higher than other treatments for birds slaughtered at 42 days of age. Finally, the results of this study demonstrate firstly that it is not possible to withdraw but it can be possible to reduce VP in finisher broiler's diets without negative effects on performance and meat quality during the time of freezing in both methods of rearing. Secondly, it is possible to reduce the VP levels in diet of broilers reared in floor system more than battery cage system.

Keywords: Broiler, cage, floor, meat lipid oxidation, vitamin premix

Introduction

Vitamins are essential for the normal growth and development of a multicellular organism. These nutrients facilitate the chemical reactions that produce among other things, skin, bone, and muscle. Even minor deficiencies may cause permanent damage. Skinner *et al*. (1992) reported that removing vitamin (V) and trace mineral (TM) premixes from broiler diets from 28 to 49 d of age had little impact on growth performance. In contrast, Deyhim and Teeter (1993) detected reduced performance for several growth and carcass traits when the same withdrawal period was examined. Baker (1997) reported that 7day removal of supplemental V and TM from broiler maize-soya bean meal diets from 35 to 42 day post hatching decreased weight gain in three different broiler strains. Maiorka *et al.* (2002) indicated that V and TM withdrawal at 42 day of age did not impair feed intake or weight gain, but significantly affected feed conversion ratio. Khajali *et al*. (2006) suggested that vitamin premix withdrawal from the finisher diet of broiler chickens did not affect the performance. There are several reports about vitamin premix (VP) withdrawal in broiler's diets based on corn and floor rearing system (Christmas *et al*., 1995; Maiorka *et al*., 2002; Khajali *et al*., 2006), but based on the following causes, it seems necessary to study the effect of withdrawal or reduction of vitamin premix in finisher diets of broilers based on wheat and barley in the battery cage system. 1) Corn is the high energy grain favored by most poultry nutritionists and poultry producers. However, it is not always available at an economic price. Wheat and barley may be a more economic and readily available alternative; There is lack of reports about removal of VP from broiler finisher diets based on wheat and barley.Also there are differences between vitamin content in wheat, barley and corn (NRC, 1994). 2)

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Birds in cages require more dietary vitamins than those on floor housing because of more limited opportunity for coprophagy (McDowell, 2000). 3) It is not clear whether the level of vitamin E in meat of broilers is sufficient for stability and meat quality after slaughter and long-term storage in freezer; because lipid oxidation is a major cause of meat quality deterioration, as products of autoxidation of unsaturated fatty acids affect wholesomeness and nutritional value (Pearson *et al*., 1983).

The purpose of the present study was to investigate the effects of reduction or withdrawal of the VP from broiler diets based on wheat and barley during finisher period on performance and meat quality in floor and battery cage broiler raising systems.

Materials and methods

Animals, Allotment, and Diets:

In both experiments procedures adopted in the study were in accordance with animal welfare norms. Both experiments were conducted to compare different levels of VP on broiler finisher diets at 29 to 42 days of age on performance and meat quality in floor and battery cage rearing systems in one common poultry house. The average initial body weight of chicks (1-day old Ross 308) in each pen was 42 g. Room temperature was kept at 34°C during the first 3 days of the experiment and then reduced gradually according to age until reaching 22°C at 21 d. The light was continuous during the first 3 d, after which the lighting regimen was set at 23 h/d. Feed intake and body weight gain were measured at the end of each week of finisher period and feed conversion ratio was calculated. The ingredient composition of the experimental diets and the nutrient composition are shown in (Table 1). The diets in all treatments were based on wheat and barley with different levels and access time to VP as follows: T1) the basal diet without VP during 29-42 days; T2) the basal diet with 33% VP during 29-42 days; T3) the basal diet with 33% VP during 29-35 days and without it during 36-42 days; T4) the basal diet with 66% VP during 29-42 days; T5) the basal diet with 66% VP during 29-35 days and without it during 36-42 days; T6) the basal diet with 100% VP during 29-42 days as control group and T7) the basal diet with 100% VP during 29-35 days and without it during 36-42 days (Table 2).

Table 1. Composition of the starter and grower diets used in the pre-experimental

Ingredient (%)	Starter diet (g/kg)	Grower diet (g/kg)
Wheat	34.00	35.14
Barely	32.00	30.00
Soya bean meal (440 g/kg CP)	23.98	26.93
Corn gluten meal	5.62	2.51
Soya oil	1.03	1.78
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	0.06	0.21
L-Lysine-HCl	0.13	0.26
Multi Enzyme (Rovabio®)[3]	0.05	0.05
Calculated compositions		
ME (kcal/kg)	2850.0	2860.0
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

12.5 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.

22.5 kg of trace mineral premix contained: 100g Mn, 50g Fe, 100g Zn, 10g Cu, 1g I, 200mg Se.

3. This enzyme 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 .

Mash feed and water were available for ad-libitum consumption. Prior to formulation, all major dietary ingredients were analyzed for AMEn, amino acid (AA) profiles (according to prediction formula existing in NRC (1994), crude protein (CP), crude fiber (CF) and ether extract (EE) as described by AOAC (2000). The total nitrogen (N) content was measured by the Kjeldahl method (Kjeltec 1030 Autoanalyzer, Foss Tecator AB, Hogans, Sweden).CP was calculated as N×6.25. The crude fiber (CF) content was determined using an automated Fibertech Foss Tecator 1010 apparatus (AOAC 2000, ID 944.05). The ash ether extract (EE) content was determined by the Soxtec automated apparatus (Soxtec system, HT, Foss Tecator 1043) (AOAC 2000, ID 920.39).

Table 2. Compositions of the diets used during the experimental period (29 through 42 d of age) in experiment 1 and 2.

Ingredients (%)	Treatment1*	Treatment2	Treatment4	Treatment6
Wheat	36.38	36.05	35.97	35.79
Barely	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[1]	0.00	0.08	0.16	0.25
Trace mineral premix[2]	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-HCl	0.07	0.07	0.07	0.07
Multi Enzyme (Rovabio®)	0.05	0.05	0.05	0.05
Total	100	100	100	100
Calculated compositions				
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
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

*T1) the basal diet with no VP at 29-42 days; T2) 33% VP during 29-42 days; T4) 66% VP at 29-42 days and T6) 100% VP at 29-42 days. Treatments 3, 5 and 7 at age 35 days, respectively, and treatments 2, 4 and 6 at age 36 days after treatment as a diet had no VP.

[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.

[2] 2.5 kg of trace mineral premix contained: 100g Mn, 50g Fe, 100g Zn, 10g Cu, 1g I, 200mg Se.

Distribution of chicks in floor system (Experiment 1) and battery cage system (Experiment 2) were as follows:

Experiment 1:

A total of 840 male broiler chicks (Ross 308) were used in this experiment. Chicks were raised until 29 day of age and weighed (1130±13.6 g), then distributed into pens in a completely randomized block design with seven treatments with four repetitions per treatments and 30 broiler chicks per floor pen replicate.

Experiment 2:

A total of420 male broiler chicks were distributed with similar average body weight into battery cages at 23 day of age and fed with similar grower diet up to 28 day of age. They were then distributed into cages in a completely randomized block design with seven treatments and 15 broiler chicks per cage replicate. Initial body weights were similar in all cages (1125±11.1 g).

Thiobarbituric acid value:

Four thigh meat samples from each replicate in experiment 1 and two thigh meat sample from each replicate in experiment 2 in two slaughters (35 and 42 days) were stored in separate oxygen permeable plastic bags at -20 °C until required for chemical analysis from 180 days. The extent of lipid oxidation in thighs was assessed by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by AOCS

(2009), using third derivative spectrophotometry. The height of the third-order derivative peak that appeared at approximately 521.5 nm was used for calculation of the MDA concentration in the samples. Tetrathoxypropane (Sigma, St. Louis, MO) was used as an MDA precursor in the standard curve. TBARS was expressed as micrograms of MDA per kilogram of sample.

Blood chemistry:

Blood from 4 birds in each group (8 from each replicate experiment 1 and 2) at 35 and 42 days of ages were collected by heart puncture using Vaccutainer tubes (BD Bioscience, Franklin Lakes, NJ) for serum clinical chemistry. Blood chemistry was determined using a clinical chemistry analyzer (Chiron Corporation, San Jose, CA). Aspartate aminotransferase (AST) was assayed as the markers of muscle, bone or liver damage (Rath et al., 2007).
Statistical Analysis: The data was subjected to ANOVA as a completely randomized block design using the GLM procedure of SAS (2002) software. Means were separated by Duncan's Multiple Rang Test at significance level of $P<0.05$.

Results

Animal Performance:

Mortality for all groups was within the expected range and there was no significant difference in mortality for all treatments. The feed intake (FI), body weight gain (BWG) or feed conversion ratio (FCR) of chicks fed with the different experimental diets for both experiments at 29-35 days of age are shown in Table 3. There were no differences in FI, BWG or FCR between the dietary treatments for either of experiment at 29-35 days of age ($P > 0.05$). The results of experiment 1 and 2 for FI, BWG or FCR at 36-42 days of age are shown in Table 4. There were no significant differences among the treatments with respect to FI, BWG or FCR in experiment 1 at 36-42 days of age ($P > 0.05$). But the results of experiment 2 demonstrated that performance was affected by VP withdrawal; broilers fed with a diet without VP (T1) at 36-42 days of age had poorer performance as compared to those receiving the VP. Vitamin premix reduction or withdrawal at different ages did not significantly affect FI, BWG or FCR in experiment 1 at 29-42 days of age ($P > 0.05$; Table 5). Although the results of experiment 2 demonstrated that FI, BWG and FCR were affected by VP withdrawal, broilers fed with a diet without VP (T1) at 36-42 days of age had poorer performance as compared to those receiving the VP ($P < 0.05$; Table 5).

Lipid Oxidation in Thigh Meat and ALP: The content of TBARS in the thigh meat samples of treatments for experiment 1 and 2 after 6 month storage in freezer are shown in figures 1, 2, 3 and 4. The results of TBARS values for experiment 1 showed there were no significant differences between TBARS values of thigh meat samples for birds slaughtered at 35 days of age (Figure 1). However, TBARS values of treatment without VP were significantly higher than other treatments ($P < 0.05$) in the birds that slaughtered at 42 days of age (Figure 2). The results of TBARS values for experiment 2 showed there were significant differences ($P < 0.05$) between TBARS values of T1 and other treatments at 35 days of age (Figure 3), The

Table 3. VP reduction or withdrawal effects on performance at 29-35 days of age

	Feed intake (g/day)		Body weight gain (g/day)		Feed conversion ratio (g/g)	
T	E1*	E2*	E1	E2	E1	E2
1	1017.5	968.7	595.8	565.2	1.71	1.71
2	1002.5	976.6	580.1	557.6	1.73	1.75
3	1012.3	975.8	607.5	577.3	1.67	1.69
4	1018.9	977.1	600.7	568.9	1.70	1.72
5	1025.9	978.6	602.6	579.2	1.70	1.69
6	1016.0	974.6	611.1	582.7	1.66	1.67
7	1021.02	979.8	608.5	579.7	1.68	1.69
SEM	21.8	17.64	17.52	32.41	0.05	0.05

*E1: Experiment 1 and E2: Experiment 2

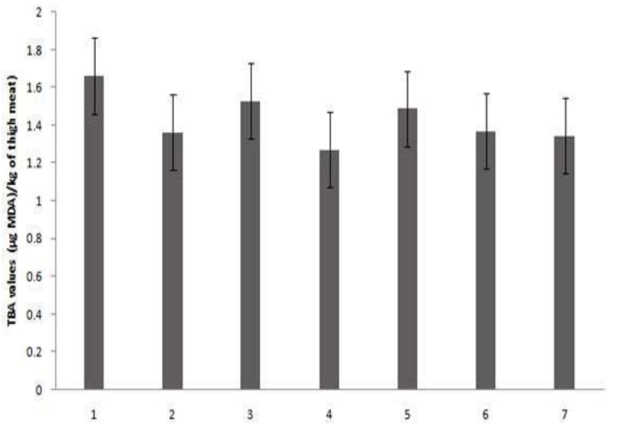

Fig. 1. Influence of VP reduction or withdrawal on thiobarbituric acid reactive substance (TBARS) values in thigh meat [µg of malondialdehyde (MDA)/kg on a dry matter basis} from the dietary treatment with 61 g of polyunsaturated fatty acids (PUPA)/kg of feed and non supplemented with a•tocopheryl acetate at slaughtered 35 day of age in experiment I.

TBARS values of T1 and T3 were significantly higher ($P<0.05$) than other treatments of thigh meat samples for birds slaughtered at 42 days of age (Figure 4). Also, The TBARS values for T2 were significantly higher ($P<0.05$) than for ther treatments (Figure 4). The results of experiment 1 showed that vitamin premix reduction and withdrawal at 29 d of age did not impair ALP during the final period of broiler chicken (Table 6). However, the results of experiment 2 showed that there were significant differences ($P < 0.05$) between the T1 and other treatments for ALP in 29-35 and 36-42 days of age (Table 6).

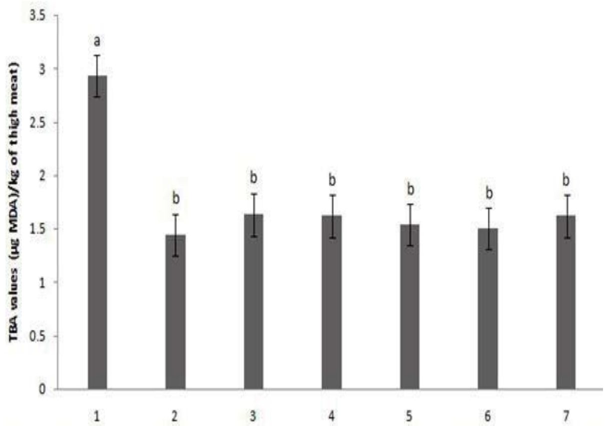

Fig. 2. Influence of VP reduction or withdrawal on thiobarbituric acid reactive substance (TBARS) values in thigh meat [µg of malondialdehyde (MDA)/kg on a dry matter basis} from the dietary treatment with 61 g of polyunsaturated fatty acids (PUPA)/kg of feed and non supplemented with a•tocopheryl acetate at slaughtered 42 day of age in experiment I.

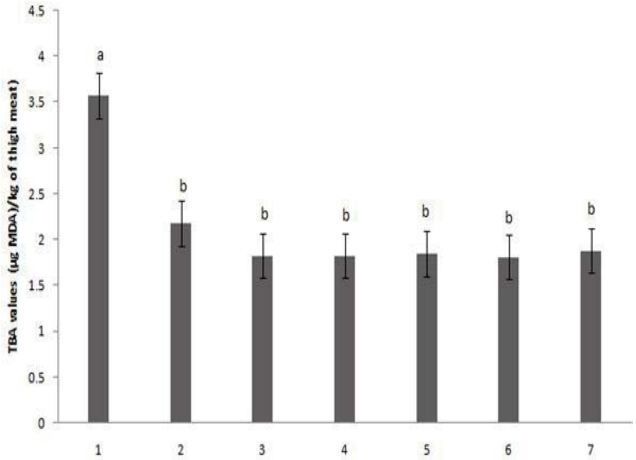

Fig. 3. Influence of VP reduction or withdrawal on thiobarbituric acid reactive substance (TBARS) values in thigh meat [µg of malondialdehyde (MDA)/kg on a dry matter basis} from the dietary treatment with 61 g of polyunsaturated fatty acids (PUPA)/kg of feed and non supplemented with a•tocopheryl acetate at slaughtered 35 day of age in experiment 2.

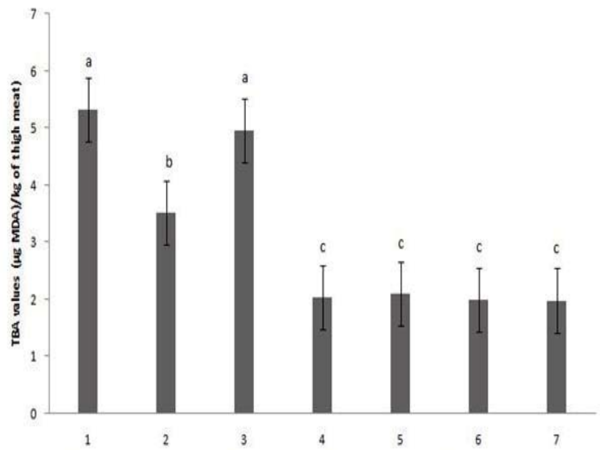

Fig. 4. Influence of VP reduction or withdrawal on thiobarbituric acid reactive substance (TBARS) values in thigh meat [µg of malondialdehyde (MDA)/kg on a dry matter basis} from the dietary treatment with 61 g of polyunsaturated fatty acids (PUPA)/kg of feed and non supplemented with a•tocopheryl acetate at slaughtered 42 day of age in experiment 2.

Discussion

Animal Performance: The findings of this study in experiment 1 were similar to those reported by Skinner *et al.* (1992) and Maiorka *et al.* (2002), as they showed that vitamin and mineral premix withdrawal from the finisher diet of broiler chickens did not affect performance. Skinner *et al.* (1992) suggested that the lack of a withdrawal effect could be related to the availability in the body of vitamins

Table 4. Vitamin premix reduction or withdrawal effects on performance at 36-42 days of age

	Feed intake (g/day)		Body weight gain (g/day)		Feed conversion ratio (g/g)	
T	E1	E2	E1	E2	E1	E2
1	1204.1^{a}	1076.8^{bc}	640.0^{a}	445.1^{bc}	1.88^{a}	2.42^{bc}
2	1183.5^{a}	1223.4^{bd}	620.6^{a}	654.2^{bd}	1.91^{a}	1.87^{bd}
3	1181.3^{a}	1229.5^{bd}	605.3^{a}	637.6^{bd}	1.95^{a}	1.93^{bd}
4	1185.8^{a}	1204.3^{bd}	672.3^{a}	663.0^{bd}	1.76^{a}	1.82^{bd}
5	1183.4^{a}	1239.9^{bd}	655.1^{a}	678.4^{bd}	1.81^{a}	1.83^{bd}
6	1203.2^{a}	1257.5^{bd}	654.7^{a}	684.8^{bd}	1.84^{a}	1.84^{bd}
7	1199.0^{a}	1253.0^{bd}	648.8^{a}	679.2^{bd}	1.85^{a}	1.84^{bd}
SEM	20.06	35.77	35.06	30.24	0.07	0.1

a,bMeans within rows with different superscript letters are different (P <0.05).
c,dMeans within columns with different superscript letters are different (P <0.05).

Table 5. Vitamin premix reduction or withdrawal effects on performance at 29-42 days of age

	Feed intake (g/day)		Body weight gain (g/day)		Feed conversion ratio (g/g)	
T	E1	E2	E1	E2	E1	E2
1	2222.2^{a}	2048.6^{c}	1280.0^{a}	1010.4^{bc}	1.74^{a}	2.03^{bc}
2	2186.2^{a}	2199.9^{d}	1241.2^{a}	1212.0^{bd}	1.76^{a}	1.82^{bd}
3	2193.7^{a}	2205.3^{d}	1210.6^{a}	1075.0^{bd}	1.81^{a}	2.05^{bd}
4	2204.6^{a}	2181.3^{d}	1344.6^{a}	1231.9^{bd}	1.64^{a}	1.77^{bd}
5	2209.3^{a}	2218.4^{d}	1310.3^{a}	1253.3^{bd}	1.69^{a}	1.77^{bd}
6	2219.3^{a}	2232.2^{d}	1309.4^{a}	1267.4^{bd}	1.69^{a}	1.76^{bd}
7	2220.0^{a}	2232.9^{d}	1297.7^{a}	1259.2^{bd}	1.71^{a}	1.77^{bd}
SEM	14.56	23.28	21.02	32.9	0.04	0.04

a,bMeans within rows with different superscript letters are different (P <0.05).
c,dMeans within columns with different superscript letters are different (P <0.05).

Table 6- Aspartate aminotransferase (103U/L) of birds fed different vitamin premix at 35 and 42 days

T	ALP E1 35 day	ALP E2 35 day	ALP E1 42 day	ALP E2 42 day
1	20.10^{a}	35.20^{bc}	29.20^{a}	76.10^{bc}
2	20.05^{a}	23.23^{ad}	27.38^{a}	30.20^{ad}
3	20.03^{a}	21.56^{ad}	25.23^{a}	30.42^{ad}
4	20.08^{a}	20.65^{ad}	28.63^{a}	28.13^{ad}
5	19.45^{a}	20.52^{ad}	28.53^{a}	26.11^{ad}
6	19.18^{a}	20.15^{ad}	25.45^{a}	28.68^{ad}
7	19.96^{a}	20.12^{ad}	25.65^{a}	26.05^{ad}
SEM	1.65	4.65	3.95	5.89

a,bMeans within rows with different superscript letters are different (P <0.05).
c,dMeans within columns with different superscript letters are different (P <0.05).

and minerals for further growth, as the amount of these supplements usually exceeds two or three times the recommended broiler chicken requirement in poultry diets. In opposition, omitting vitamin from the finisher diet for the same removal period decreased weight gain in three different broiler strains (Deyhim and Teeter, 1993; Patel *et al.*, 1997). These differences may be due to the type of rearing system (floor litter or cages) or differences in diet composition.

The findings in experiment 2 differ slightly from those reported by Skinner *et al.* (1992) and Khajali *et al.* (2006) as they showed that VP withdrawal from the finisher diet of broiler chickens did not affect performance. But the findings of this study were close to reports of Deyhim and Teeter (1993). They also demonstrated that broiler chickens reared in batteries under a cycling ambient temperature (24°C to 35°C, creating heat stress), and fed with diets without vitamin and mineral premix had reduced weight gain and poorer feed conversion as compared to birds fed normally with supplemented diets. Whereas birds in battery cage systems require more dietary vitamins than those on floor housing because of more limited opportunity for coprophagy (McDowell, 2000).

Based on these results it seems that, it can be possible to withdraw VP in the finisher broiler diets, while VP can be reduced in the finisher broiler diets (until 33%) in 29-35 days of age and then removed during 36-42 days of age (T3), without a significant negative effect on performance of broilers.

Lipid Oxidation in Thigh Meat and ALP: It is generally believed that lipid oxidation in muscle foods is initiated in the highly unsaturated phospholipid fraction in subcellular membranes (Gray and Pearson, 1987). The autocatalytic peroxidation process probably begins immediately after slaughter. The biochemical changes that accompany post-slaughter metabolism and post-mortem ageing in the conversion of muscle to meat give rise to conditions where the process of lipid oxidation is no longer tightly controlled and the balance of pro-oxidative factors/antioxidative capacity favours oxidation. There is now considerable interest in the antioxidant properties of naturally occurring substances such as vitamin E (Loliger, 1991; Nockels *et al.*, 1996). Vitamin E, which is usually incorporated in the diet as a-tocopheryl acetate, constitutes the second line of antioxidant defense in biological systems, and is the major lipid-soluble antioxidant, breaking the chain of lipid peroxidation in cell membranes and preventing the formation of lipid hydroperoxides (Halliwell, 1987). Hence it seems that the birds fed by finisher diets containing 33% and 66% VP at 29-36 days of age in experiments 1 and 2 respectively, can access to enough vitamin E for prevention of meat damage during the time of freezing. Therefore, based on the results of TBARS values, it is necessary to consider levels of vitamin E in finisher broiler diets for save meat quality. Muscular damage as a result of vitamin E deficiency causes leakage of intercellular contents into the blood. Thus, elevated levels of aspartate amino transferase enzyme (above the normal concentrations for particular species) serve as diagnostic aids in detecting tissues degeneration. A distinction regarding type of tissue degeneration can sometimes be made. Enzyme test are very sensitive, and an elevation of enzyme activity in serum is usually discovered before any pathological changes or clinical sings appear (McDwell, 2000).

So it seems that, VP reduction can only be allowed until 33% and 66% in the finisher broiler diets at 29-36 days of age in floor system and battery cage system respectively.

Conclusion

The results of this study demonstrated that on the one hand, it is not possible to withdraw but it can be possible to reduce vitamin premix in finisher broiler's diets without negative effects on performance and meat quality during the time of freezing in both methods of rearing. On the other hand, it is possible to reduce the vitamin premix levels in diet of broilers reared in floor system more than battery cage system.

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