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EFFECT OF DIFFERENT LEVELS OF VITAMIN PREMIX REDUCTION OR REMOVAL DURING FINISHER PERIOD ON IMMUNOCOMPETENCE OF BROILER CHICKENS

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Abstract

Three experiments were conducted to compare the effect of a decreasing vitamin premix amount in diets from 29 to 42 days of age on performance and immunocompetence of broiler chickens in floor (experiments 1 and 2) and battery cage (experiment 3) systems. The diets were based on corn/soybean meal (experiment 1) and wheat/barley (experiments 2 and 3). On day 34, two birds from each replicate were selected and antibody responses to inoculated sheep red blood cells were determined. The results of experiments 1 and 2 showed that vitamin premix reduction/withdrawal at 29 days of age did not impair performance during the final rearing period. However, the results of experiment 3 showed that from 29 to 42 days of age, performance of birds fed the diet without vitamin premix (T1) was significantly lower than other treatments. The results of three experiments demonstrated that immunocompetence response was not affected by treatments in the finisher period. In conclusion, the results of the present study indicated that in the battery cage system it is possible to reduce dietary vitamin premix during the finisher period but withdrawal can negatively affect performance of broilers. While in the floor system it is possible to withdraw vitamin supplements from finisher diets.

Key words: broiler, cage, corn, immunocompetence, vitamin premix, wheat

Skinner et al. (1992) and Khajali et al. (2006) reported that removing vitamin (V) and trace mineral (TM) premixes from broiler diets at 28 to 49 days 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 7-day removal of supplemental V and TM from broiler maize-soybean meal diets from 35 to 42 days posthatching decreased weight gain in three different broiler strains. Maiorka et al. (2002) indicated that V and TM withdrawal at 42 days of age did not impair feed intake or weight gain, but significantly affected feed conversion ratio. Vitamin deficiencies have been shown to suppress immunocompetence (Myrvik, 1988). Therefore, the response of the immune system needs to be considered when studying the effect of vitamin withdrawal. Deyhim and Teeter (1993) showed that removal of the V and TM premixes from broiler rations did not affect immunological competence as judged by antibody titre to sheep erythrocyte injection. Khajali et al. (2006) suggested that the vitamin and trace mineral contents of the finisher diet were sufficiently high to maintain a humoral immune response.

Because few countries have the technology to produce vitamin supplements, most countries import these products. In addition, it seems that the poultry producers are now using an extra level of vitamins in broiler diets and birds excrete the excess, which makes excreta very expensive, whereas avoiding the use of extra vitamins in poultry diets could be effective in reducing production costs. Duration of removal period, variety of diets, different levels of vitamin premix and rearing systems can be important factors in these kinds of studies. In order to investigate the possibility of reducing production costs, the purpose of the present study was to investigate the effect of vitamin premix reduction and removal from finisher diets (wheat and barley/corn and soybean meal) on growth performance and immunocompetence of broilers in two rearing systems (floor and battery cage).

Material and methods

Experiment 1

The experimental procedures adopted in the study were in accordance with animal welfare standards. A total of 720 male broilers (Ross hybrid) were obtained from a local hatchery and fed on commercial starter and grower diets that met their nutrient requirements (NRC, 1994; Table 1). At 29 days of age, following a 6-h fast, chickens were weighed, wing-banded, and allotted to 36 floor pens. Initial body weights were similar in all pens (1125±9.6 g). Four dietary (based on corn/soybean meal) treatments were used: T1) the basal diet without vitamin premix from 29 to 42 days; T2) the basal diet with 33% vitamin premix from 29 to 42 days (0.8 g/kg); T3) the basal diet with 66% vitamin premix from 29 to 42 days (1.65 g/kg) and T4) the basal diet with 100% vitamin premix from 29 to 42 days (2.5 g/kg).

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

Ingredient	Starter diet (g/kg)	Grower diet (g/kg)	Finisher diet (g/kg)
Corn	575	665	670.2
Soybean meal (440 g/kg CP)	360	283	275.9
Gluten meal	18	15	
Soybean oil	20	5	26.8
Oyster shell	13	13	8.2
Dicalcium phosphate	20	12	18.5
Vitamin premix[1]	2.5	2.5	2.5
Trace mineral premix[2]	2.5	2.5	2.5
Sodium chloride	4	3	3
DL-Methionine	1		1.6
L-Lysine-HCl	0.2		0.8
Calculated composition			
AME (kcal/kg)	2950.00	2960.00	3000.00
Analyses CP (g/kg)	215	210	207
Met (g/kg)	4.1	3.7	3.4
Met + Cys (g/kg)	8.2	6.5	5.8
Lys (g/kg)	11.5	9.1	8.5

[1]Supplied per kg of diet: retinol acetate – 1100 mg, cholecalciferol – 160 µg, DL-alpha-tocopherol acetate – 10 mg, menadione – 1 mg, thiamine mononitrate – 2.3 mg, riboflavin – 5 mg, pyridoxine hydrochloride – 3 mg, cyanocobalamin – 15 µg, D-biotin – 12 mg, niacin – 12 mg, pantothenic acid – 30 mg, folic acid – 15 mg, choline chloride – 300 g.

[2]Supplied per kg of diet: iron – 350 mg, copper – 50 mg, manganese – 280 mg, cobalt – 5 mg, zinc – 250 mg, iodine – 3 mg, selenium – 200 mg, molybdenum – 0.5 mg.

Experiment 2

Two hundred and eighty-eight Ross 308 male chicks were used in this trial. Chicks were raised until 29 days of age, as described in the general procedure, weighed (1130±13.6 g), and distributed into floor pens in a completely randomized design with 4 treatments (similar levels of vitamin premix to experiment 1) with four repetitions per treatment and 18 birds per floor pen replicate. The diets in all treatments were based on wheat and barley (Table 2).

Experiment 3

Sixty-four male broiler chicks were distributed with similar average body weight into battery cages at 23 days of age and fed similar grower diet (Table 2) up to 28 days of age and distributed into cages in a completely randomized design with four treatments (similar treatments to experiment 2) with four repetitions per treatment of 4 birds each. Initial body weights were similar in all cages (1125±11.1 g).

Table 2. Composition of the starter and grower diets used in pre-experiments 2 and 3

Ingredient	Starter diet (g/kg)	Grower diet (g/kg)	Finisher diet (g/kg)
Wheat	340.0	351.4	357.9
Barley	320.0	300.0	300.0
Soybean meal (440 g/kg CP)	239.8	269.3	280.9
Gluten meal	56.2	25.1	-
Soybean oil	10.3	17.8	29.0
Oyster shell	13.0	12.9	12.4
Dicalcium phosphate	10.5	10.5	9.0
Vitamin premix[1]	2.5	2.5	2.5
Trace mineral premix[2]	2.5	2.5	2.5
Sodium chloride	2.8	2.8	2.8
DL-Methionine	0.6	2.1	1.8
L-Lysine-HCl	1.3	2.6	0.7
Multi Enzyme (Rovabio®)[3]	0.5	0.5	0.5
Calculated composition			
AME, kcal/kg	2950.00	2960.00	3000.00
Analyses CP (g/kg)	215	210	207
Met (g/kg)	4.1	3.7	3.4
Met + Cys (g/kg)	8.2	6.5	5.8
Lys (g/kg)	11.5	9.1	8.5

[1]Supplied per kg of diet: retinol acetate – 1100 mg, cholecalciferol – 160 µg, DL-alpha-tocopherol acetate – 10 mg, menadione – 1 mg, thiamine mononitrate – 2.3 mg, riboflavin – 5 mg, pyridoxine hydrochloride – 3 mg, cyanocobalamin – 15 µg, D-biotin – 12 mg, niacin – 12 mg, pantothenic acid – 30 mg, folic acid – 5 mg, choline chloride – 300 g.

[2]Supplied per kg of diet: iron – 350 mg, copper – 50 mg, manganese – 280 mg, cobalt – 5 mg, zinc – 250 mg, iodine – 3 mg, selenium – 200 mg, molybdenum – 0.5 mg.

[3]These enzymes 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.

All diets (experiments 1, 2 and 3) were fed from 29 to 42 days of age. Feed and water were available for *ad libitum* consumption.

Performance of chickens

Mortality from days 29 to 42 was determined for each pen. Body weight gain (BWG) and average daily feed intake (ADFI) of chickens were determined at 42 days of age, and then feed conversion ratio was calculated from these data.

Lymphoid organs weight

At 42 days of age, two birds of each replicate in experiments 1, 2 and 3, were selected at initial body weights similar in replicate, subjected to 6 h fasting, reweighed, then slaughtered and eviscerated in order to determine carcass yield and relative weights of lymphoid organs (bursa of Fabricius and spleen) were measured to the nearest 0.01 g.

Immunological studies

Sheep red blood cells were used as T-dependent antigens to quantify the antibody response. In the trials, two birds were selected from each of the replicated groups at 34 days of age and were inoculated intravenously with 0.1 ml of a 1% suspension of SRBC (Sheep Red Blood Cells). Blood samples were collected from the branchial vein 7 days after each injection. The serum from each sample was collected, heat inactivated at 56°C for 30 min and then analysed for total, mercaptoethanol[1]-sensitive (MES) IgM and mercaptoethanol-resistant IgG anti-SRBC antibodies as previously described (Delhanty and Solomon, 1966; Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 μL of serum was added in an equal amount of phosphate-buffered saline (PBS; pH 7.6) in the first column of a 96-well v-shaped bottom plate[2], and the solution was incubated for 30 min at 37°C. A serial dilution was then made (1:2), and 50 μL of 2% SRBC suspension was added to each well. Total antibody titres were then read after 30 min of incubation at 37°C. The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titre for agglutination. For MES (IgM) response, 50 μL of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the aforementioned procedure. The difference between the total and the IgG response was considered to be equal to the IgM antibody level (Cheema et al., 2003).

Statistical analysis

The data were subjected to ANOVA as a completely randomized design using the GLM procedure of SAS software (SAS Institute, 2002). Anti-SRBC titres and lymphoid organ weights data are not normally distributed; therefore, these data were transformed to log2 and arc sin, respectively, to normalize it by transformation. Means were separated by Duncan's multiple range test at significance level of $P<0.05$.

Results

Mortality for all groups was within the expected range and there was no significant difference in mortality for all treatments. The performance results from experiments 1, 2 and 3 for average daily feed intake (ADFI), body weight gain (BWG), and feed conversion ratio (FCR) are shown in Table 3. The results of experiments 1 and 2 showed that vitamin premix reduction and withdrawal at 29 days of age did not impair performance during the final period (days 29–42) of broiler chicken rearing ($P>0.05$). Whereas the results of experiment 3 showed that from 29 to 42 days of

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[1]Sigma Chemical Co., St. Louis, MO.

[2] Corning, Corning, NY.

age, performance of birds fed the diet without vitamin premix (T1) was significantly lower than for other treatments (P<0.05). Reduction and removal of the dietary vitamin premix in experiments 1 and 2 from the ration between days 29 and 42 had no significant effect on carcass yield (Table 4). However, removing the dietary vitamin premix from the ration between 29 and 42 days of age had a significant effect on carcass yield (Table 4; P<0.05). The bursa of Fabricius and spleen weights were not significantly different in chicks fed diets with various levels of vitamin premix in experiments 1, 2 and 3 (Table 4). In three experiments, humoral immunocompetence response (IgM, IgG and anti-SRBC titres) were not affected by different treatments (Table 5).

Table 3. Average daily feed intake (ADFI) (g), body weight gain (BWG) (g), and feed conversion ratio (FCR) (g/g) of broiler chickens from 29 to 42 days of age when premix vitamins were reduced or withdrawn; experiments (Exp) 1, 2 and 3

Treatments[1]	Exp 1			Exp 2			Exp 3		
	ADFI	BWG	FCR	ADFI	BWG	FCR	ADFI	BWG	FCR
T 1	156.45	86.32	1.81	158.73	88.27	1.80	146.33 b	72.17 b	2.03 b
T 2	157.32	88.17	1.78	156.16	85.87	1.82	157.14 a	86.57 a	1.82 a
T 3	157.12	88.54	1.77	157.47	90.93	1.73	155.81 a	87.99 a	1.77 a
T 4	159.30	90.65	1.76	158.52	90.42	1.75	159.44 a	90.53 a	1.76 a
SEM	2.74	3.25	0.05	2.56	3.02	0.04	4.52	2.35	0.05

[1](1) without vitamin premix, days 29–42; (2) 33% vitamin premix, days 29–42; (3) 66% vitamin premix, days 29–42; (4) 100% vitamin premix, days 29–42.

a, b – Means with different superscripts in each column are significantly different (P<0.05).

Table 4. Vitamin premix reduction or withdrawal effects on carcass yield and lymphoid organs weight

Treatments	Exp 1			Exp 2			Exp 3		
	carcass yield (g)	bursa (g)	spleen (g)	carcass yield (g)	bursa (g)	spleen (g)	carcass yield (g)	bursa (g)	spleen (g)
T 1	2032	2.64	1.84	2011	2.50	1.85	1730 b	2.39	2.06
T 2	2045	2.58	2.01	2051	2.58	2.08	2009 a	2.47	2.10
T 3	2041	2.72	1.94	2034	2.78	1.89	2014 a	2.40	1.86
T 4	2054	2.67	1.90	2063	2.49	2.06	2018 a	2.61	2.15
SEM	10.05	0.08	0.06	15.45	0.09	0.08	75.39	0.13	0.16

Table 5. Vitamin premix reduction or withdrawal effects on IgG and IgM anti-SRBC titres (log2)

Treatments	Exp 1			Exp 2			Exp 3		
	SRBC	IgG	IgM	SRBC	IgG	IgM	SRBC*	IgG	IgM
T 1	6.25	2.25	4.00	7.75	2.50	5.25	6.00	2.50	3.50
T 2	7.00	3.00	4.00	5.75	2.25	3.50	5.75	2.25	3.50
T 3	6.50	2.25	4.25	7.00	2.75	4.25	6.50	2.50	4.00
T 4	6.50	3.00	3.50	6.75	2.75	4.00	7.00	2.75	4.25
SEM	0.35	0.40	0.25	1.07	0.25	0.80	0.70	0.30	0.40

Discussion

The results of experiments 1 and 2 regarding the effect of vitamin premix reduction or withdrawal on growth performance are conflicting. For instance, deletion of the vitamin premix for 7 days pre-slaughter showed no decrease in growth performance of broilers (Skinner et al., 1992; Khajali et al., 2006). Conversely, omitting vitamin premix 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; Maiorka et al., 2002). These differences may be due to the type of rearing system (floor litter or cages) or differences in diet composition. The results of carcass yield weight in experiments 1 and 2 confirm the results obtained by Maiorka et al. (2002). The findings (results of experiment 3) of this study differ slightly from those reported by Khajali et al. (2006) as they showed that vitamin premix withdrawal from the finisher diet of broiler chickens did not affect body weight gain. But the findings of this study were nearly similar to reports of Deyhim and Teeter (1993). They also demonstrated that broiler chickens reared in batteries under a cycling ambient temperature (24 to 35°C, creating heat stress), and fed diets without vitamin and mineral premix had reduced weight gain and poorer feed conversion as compared to birds fed normally supplemented diets. Whereas birds in cages require more dietary vitamins than those on floor housing because of more limited opportunity for coprophagy. In fact, floor-reared broilers can access their feces to reach some vitamins, which have departed from the intestine (this is not possible for the birds that are reared in the battery cage system).

The results of carcass yield in experiment 3 confirm the results obtained by Patel et al. (1997). It should be emphasized that removal of vitamin premix from broiler diets does not imply that such diets are devoid of these essential nutrients. Unfortified diets, especially those that contain some animal protein feedstuff, may contain quantities of vitamins sufficient to meet or exceed minimum recommended needs. Vitamin premixes used in the commercial broiler industry typically provide vitamin premix at two to fourfold or more of the minimum recommended levels (Gwyther et al., 1992); thus, some storage within the carcass should be expected, especially for the fat-soluble vitamins. Under commercial growing conditions, using practical feedstuffs, it may be difficult to produce vitamin premix deficiencies in birds during the finishing period following adequate supplementation early in the growing period. Reduction of these supplements in diets fed from 29 to 42 days of age could significantly reduce growing costs with no adverse effects on performance.

Immunocompetence

Research regarding the effect of vitamin nutrition on the immunological response of avian species has been limited to vitamin E. The effect of vitamin E on immunocompetence of chickens is well known (Muir et al., 2002). In practice, nutritionists do not take into account the vitamin premix supplied from the natural feedstuffs. Consequently, the bird's requirements for these nutrients are possibly met from natural feedstuffs as well as from body reserves during a short-term vitamin withdrawal. It has been demonstrated that the immune system has a higher priority for circulating nutrients and is able to compete favourably with other tissues when nutrient levels are low (Klasing, 1998). These results are in accordance with those reported by Deyhim and Teeter (1993) and Khajali et al. (2006). These findings suggest that the vitamin contents of the finishing diet were sufficiently high to maintain a humoral immune response.

In conclusion, the results of the present study indicated that in the battery cage system it is possible to reduce dietary vitamin premix during finisher period but withdrawal can negatively affect performance of broilers. While in the floor system it is possible to withdraw vitamin supplements from finisher diets.

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Wpływ zastosowania różnych poziomów premiksu witaminowego w ostatnim okresie odchowu na immunokompetencję brojlerów kurzych

STRESZCZENIE

W trzech eksperymentach porównano wpływ zastosowania malejących ilości premiksu witaminowego w dietach w okresie od 29. do 42. dnia życia na użytkowość i immunokompetencję brojlerów kurzych utrzymywanych w chowie podłogowym (dośw. 1 i 2) i bateryjnym (dośw. 3). Podstawą diet były kukurydza/mączka sojowa (dośw. 1) oraz pszenica/jęczmień (dośw. 2 i 3). W 34. dniu życia z każdego powtórzenia wybierano dwa brojlery w celu określenia odpowiedzi przeciwciał na wszczepienie erytrocytów owczych. Wyniki doświadczeń 1 i 2 pokazały, że zmniejszenie/wycofanie premiksu witaminowego w 29. dniu życia nie pogorszyło użytkowości w końcowym okresie odchowu. Wyniki doświadczenia 3 wykazały jednak, że od 29. do 42. dnia życia użytkowość ptaków żywionych dietą bez premiksu witaminowego (T1) była istotnie niższa w porównaniu do innych grup. Rezultaty trzech doświadczeń wskazują, że diety zastosowane w końcowym okresie odchowu nie miały wpływu na odpowiedź immunologiczną. Wyniki obecnego eksperymentu wskazują, że w chowie bateryjnym można zmniejszyć poziom premiksu witaminowego w diecie w końcowym okresie odchowu brojlerów, jednak całkowite jego wycofanie może niekorzystnie wpłynąć na ich użytkowość. Dodatki witaminowe można natomiast wycofać z diet typu finiszer brojlerów utrzymywanych w systemie podłogowym.