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Response to withdrawal of vitamin and trace mineral premixes from finisher diet in broiler chickens under the hot and humid tropical condition

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ABSTRACT

This study was conducted to assess the effects of omitting vitamin (VIT) and trace mineral (TRM) premixes from the finisher diet on growth performance, antibody production against Newcastle disease (ND) vaccination, leg bone strength and incidence of foot pad dermatitis (FPD) in male and female broiler chickens. Birds were raised on floor pens in a naturally ventilated house using wood shavings as litter. Commencing from day 21, equal number of male and female birds were subjected to one of four VIT and TRM premixes withdrawal treatments as follows: (i) withdrawal from 21 to 42 days, (ii) withdrawal from 28 to 42 days, (iii) withdrawal from 35 to 42 days, and (iv) no withdrawal (control). In comparison to the control group, feed intake, body weights, feed conversion ratios and antibody responses to Newcastle disease vaccination were not affected by any of the supplement withdrawal treatment. Male birds had lower antibody production and higher bone-breaking strength as compared to their female counterparts. Irrespective of gender, withdrawal of VIT and TRM premixes from 21 to 42, and 28 to 42 days adversely affected bone-breaking strength. In conclusion, VIT and TRM premixes can be removed from the diet of male and female broiler chickens from 21 to 42 days without any adverse effects on growth performance, mortality, antibody response and incidence of foot pad dermatitis. However, bone-breaking strength was adversely affected by the withdrawal of VIT and TRM premixes for more than seven days.

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Introduction

The diet of commercial broiler chickens is routinely fortified with vitamin (VIT) and trace mineral (TRM) premixes. However, a portion of the required VIT and TRM may come from major feedstuffs such as maize and soybean meal. The practice of omitting both VIT and TRM premixes from the diet of broilers may substantially reduce production costs. Skinner et al. (1992), and Christmas et al. (1995) reported that the withdrawal of both premixes from 28 to 49, and 35 to 42 days of age had negligible effect on growth performance. It has also been reported that, humoral immunity is not affected by withdrawal of both VIT and TRM premixes during the finisher period (Deyhim & Teeter 1993; Khajali et al. 2006). However, the question remains, whether such practices may have an adverse effect on broilers under hot and humid tropical conditions. Heat stress is detrimental to the absorption of VIT (Klasing 1998) and may reduce plasma and tissue concentrations of TRM on poultry (Beisel 1982). Supplementation of drinking water with vitamins A, D, E and B complex has been reported to be beneficial for the performance and immune function of heat-stressed broilers (Ferket & Qureshi 1992). Sahin et al. (2002b) reported that dietary supplementation of chromium, improved feed intake, weight gain and feed efficiency of broilers exposed to high environmental temperatures. Zinc fortification was also shown to be beneficial in enhancing growth performance and carcase traits in heat-stressed broilers (Kucuk et al. 2003). Thus, there is a possibility that broilers under hot environmental conditions require further VIT and TRM fortification in their diet. There is a paucity of information and literature regarding the effects of withdrawal of dietary VIT and TRM premixes in different genders. A single available report by Skinner et al. (1992) stated that there was no gender × withdrawal interaction for growth performance. However, the existence of interactions for other parameters such as bone characteristic remains unclear. Moreover, the

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study conducted by Skinner et al. (1992) was carried out under temperate conditions and it remains to be seen what the scenario would be under tropical conditions. It has been shown that there is an association between growth rate and sensitivity to heat stress in broiler chickens (Cahaner et al. 1996). Given the importance of dietary VIT (Sahin et al. 2002a) and TRM (Sands & Smith 1999; Sahin et al. 2009) in alleviating heat stress, it is therefore possible that the omission of both premixes may result in different responses in male and female broiler chickens raised under hot and humid conditions.

Despite the importance of dietary VIT and TRM in bone metabolism and bone strength (Rath et al. 2000), there is a paucity of information on the effects of VIT and TRM premixes withdrawal on bone strength, particularly under high environmental temperatures. Under temperate conditions, Skinner et al. (1992) reported that withdrawal of both VIT and TRM premixes from the finisher diet had negligible effects on leg disorders in broilers. The present work was carried out to evaluate the effects of VIT and TRM premixes withdrawal from the finisher diet at various stages on growth performance, antibody production against vaccination, and bone strength in male and female broiler chickens raised under hot and humid tropical conditions. In the current study, we also investigated the influence of omitting VIT and TRM premixes on incidence of foot pad dermatitis (FPD). Pododermatitis is a type of contact dermatitis (Greene et al. 1985), that manifests itself in poultry as discolouration, erosions and ulcers on the plantar region of the foot (Ekstrand et al. 1997). The cause of FPD is a complex but nutritional deficiency that has been suggested as one of the possible factors of the disease (Sheperd & Fairchild 2010).

Materials and methods

Birds and housing

The study was conducted as per the guidelines of the Animal Care and Use Committee, Faculty of Agriculture, Universiti Putra Malaysia. A total of 400 male and 400 female day-old Cobb 500 broiler chicks were obtained from a local hatchery. On day 1, the chicks were weighed and randomly allocated to 40 floor pens (1.7 m × 1.4 m) using wood shavings as litter in a naturally ventilated open-housing system. Daily environmental temperatures fluctuated between 24 and 35 °C and relative humidity between 75 and 90%. The chicks received a live Newcastle disease (ND) vaccine (B1 Type, La Sota Strain, Massachusetts Type, Gainesville, FL) intraocularly on day 7 and 21. Feed and water was provided *ad libitum* and lighting was continuous.

Experimental design

The composition of experimental diets is as shown in Table 1. All birds were fed a standard starter diet (3,035 kcal of ME/kg; 21.5% CP) from 1 to 20 days and a finisher diet (3180 kcal of ME/kg; 21.5% CP) from 21 to 42 days. Commencing from day 21, equal number of male and female birds were subjected to one of the four dietary treatments with five replicate pens per diet-gender subgroup. The dietary treatments were as follows: (i) withdrawal of VIT and TRM premixes from 21 to 42 days (W3), (ii) withdrawal of VIT and TRM premixes from 28 to 42 days (W2), (iii) withdrawal of VIT and TRM premixes from 35 to 42 days (W1), and (iv) no withdrawal of VIT and TRM premixes (control). Birds of different treatment groups had similar body weight on day 21 (data not shown). Commercial VIT and TRM premixes (Lutamix Gladron 528 and Gladron Poultry Mineral; Gladron®, Malaysia) for broiler chickens were used in the present study.

Sampling and measurement

Feed intake (FI) and body weight (BW) was recorded weekly and feed conversion ratios (FCR) were calculated.

Table 1. Composition of experimental diets.

		Finisher	
Ingredient, %	Starter	Normal	No premixes
Maize	56.75	61.76	61.76
Soybean meal	35.69	29.75	29.75
Palm olein	3.81	5.42	5.42
Limestone	1.32	1.13	1.13
Salt (NaCl)	0.34	0.34	0.34
Monocalcium phosphate	1.54	1.26	1.26
Mineral premix[a]	0.10	0.10	0.00
Vitamin premix[b]	0.05	0.05	0.00
L-Lysine	0.15	0.04	0.04
DL-Methionine	0.19	0.15	0.15
Threonine	0.06	0.00	0.00
Sand	0.00	0.00	0.15
Nutrient composition, %			
ME, Kcal/kg	3035	3180	3180
Crude protein	21.50	19.00	19.00
Crude fibre	3.20	3.03	3.03
Ether extract	6.47	8.16	8.16
Ca	0.90	0.76	0.76
Available P	0.45	0.38	0.38
Lysine	1.34	1.07	1.07
Methionine	0.52	0.45	0.45
Threonine	0.88	0.73	0.73

[a]Each kg of mineral premix contained: sodium selenite 0.2 g; ferrous sulphate 80 g; manganese sulphate 100 g; zinc oxide 80 g; copper sulphate 15 g; potassium iodate 1 g; cobalt carbonate 0.25 g; potassium chloride 4 g; sodium bicarbonate 1.5g.

[b]Each kg of vitamin premix contained: retinol 15 g; cholecalciferol 0.25 g; Dl-α-tocopherol 75 g; menadione 20 g; thiamine 10 g; riboflavin 30 g; pyridoxine 20 g; cyanocobalamin 0.10 g; calcium D-pantothenate 60 g; nicotinic acid 200 g; folic acid 5 g; biotin 0.235 g.

On day 42, 16 birds per pen were randomly selected and examined and scored visually for the incidence and severity of FPD (Nagaraj et al. 2007). The footpad lesions were examined macroscopically in both feet and assigned to one of the three classes: score 0 = no lesions, score 1 = mild lesions of <1.5cm, score 2 = severe lesions of >1.5cm. The mean FPD score of the flock was calculated as the cumulative total of the lesion scores divided by the total number of examined birds.

Subsequently, three birds from each replicate pen were randomly selected, slaughtered according to the halal method (Farouk et al. 2014), and the right tibias were removed as drumsticks with flesh intact for determination of bone-breaking strength. Blood samples from each bird were collected during slaughtering, to measure antibody response against Newcastle disease vaccination.

Bone processing

The tibia processing was carried out according to previously described methods by Mutus et al. (2006). Briefly, drumsticks were placed in boiling water for 10 min, completely defleshed, dried at room temperature for 24 h and then stored in a −20 °C until further analysis. The length and weight of the thawed tibia were recorded and bone-breaking strength (TBS) was measured using an Instron 4502 material testing machine (Instron Corp., Canton, MA). Bones were sheared in the middle using a crosshead speed of 1.3 mm/min to minimise splintering. The bone weight/length ratio (robusticity index; RI) was calculated using the following formula:

robusticity index = bone length/cube root of bone weight (Reisenfeld 1972).

Robusticity index has been shown to be correlated with bone mineral content and density (Monteagudo et al. 1997). A smaller robusticity index indicates stronger bones.

Measurement of antibody response

Sera were separated from blood samples and used for the detection of ND antibodies using a commercial quantitative ELISA kit (part number: 99-09263, IDEXX laboratory, Inc., Westbrook, ME).

Statistical analysis

All the data were subjected to the ANOVA test using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Data were analysed within each gender with diet as the main effect. Diet, gender and their interactions were included as the main effects. When interactions were significant, comparisons were made within each experimental variable. When significant effects were found, comparisons among multiple means were made by Duncan's multiple range test. Mortality data were analysed using the chi-square test. Significance level is considered at $p \leq .05$.

Results and discussion

Growth performance

The omission of both VIT and TRM premixes from the finisher diet at various periods did not affect BW, FI and FCR irrespective of gender ($p = .468$, $p = .744$ and $p = .885$, respectively), as shown in Table 2. The results corroborate with those of Skinner et al. (1992), Khajali et al. (2006), and Moravej et al. (2012b, 2013) who observed that the growth performance of broilers was

Table 2. Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) and mortality of broilers fed diets with or without supplementation of vitamin (VIT) and mineral (TRM) premixes at 1 to 42 days.

Treatment	BW, g	FI, g	FCR	Mortality, %
Diet				
W3	2331 ± 59	4320 ± 92	1.86 ± 0.02	9.00
W2	2325 ± 48	4315 ± 69	1.86 ± 0.03	9.50
W1	2404 ± 53	4399 ± 97	1.83 ± 0.03	12.50
Control	2379 ± 64	4383 ± 90	1.85 ± 0.02	7.03
Gender				
Male	2479[a] ± 31	4538[a] ± 43	1.83 ± 0.02	9.50
Female	2241[b] ± 26	4170[b] ± 45	1.86 ± 0.02	9.52
p-values				
Diet	.468	.744	.885	.540
Gender	<.001	<.001	.271	.688
Diet × Gender	.492	.951	.434	.953

Mean ± SEM bearing uncommon superscript within a column-subgroup are significantly different.
W3: VIT and TRM premixes withdrawal from 21 to 42 days; W2: VIT and TRM premixes withdrawal from 28 to 42 days; W1: VIT and TRM premixes withdrawal from 35 to 42 days; Control: No VIT and TRM premixes withdrawal.

Table 3. Tibia breaking strength (TBS) and robusticity index (RI) and foot-pad dermatitis (FPD) and antibody response of broilers fed diets with or without supplementation of vitamin (VIT) and mineral (TRM) premixes at 42 days of age.

Treatment	TBS (*N*)	RI	FPD	Antibody response (log10)
Diet				
W3	$244.82^{c} \pm 14.11$	$4.87^{a} \pm 0.07$	0.36 ± 0.07	$2.90^{b} \pm 0.07$
W2	$279.95^{bc} \pm 16.95$	$4.89^{a} \pm 0.08$	0.43 ± 0.08	$2.90^{b} \pm 0.07$
W1	$310.89^{ab} \pm 19.04$	$4.75^{ab} \pm 0.04$	0.30 ± 0.07	$3.14^{a} \pm 0.07$
Control	$354.00^{a} \pm 18.75$	$4.67^{b} \pm 0.05$	0.46 ± 0.07	$3.02^{ab} \pm 0.06$
Gender				
Male	$321.30^{a} \pm 13.27$	$4.68^{b} \pm 0.03$	0.47 ± 0.06	$2.88^{b} \pm 0.04$
Female	$272.76^{b} \pm 12.94$	$4.91^{a} \pm 0.05$	0.34 ± 0.05	$3.09^{a} \pm 0.05$
p-values				
Diet	.001	.009	.466	.040
Gender	.005	<.001	.101	.003
Diet × Gender	.428	.013	.201	.547

Mean ± SEM bearing uncommon superscript within a column-subgroup are significantly different.
W3: VIT and TRM premixes withdrawal from 21 to 42 days; W2: VIT and TRM premixes withdrawal from 28 to 42 days; W1: VIT and TRM premixes withdrawal from 35 to 42 days; Control: No VIT and TRM premixes withdrawal; N = Newton.

not adversely affected by the withdrawal of VIT and TRM premixes from the finisher diet. In terms of growth performance, the present findings also suggest that both male and female broilers responded similarly to VIT and TRM premixes withdrawal from day 21 to 42. Similarly, Skinner et al. (1992) indicated a lack of gender × diet interactions for body weight, feed intake and feed efficiency in broilers deprived of VIT and TRM premixes from 42 to 49 days of age. The results obtained in this study as well as previous studies suggest that the VIT and TRM from maize, soybean meal and body reserves are sufficient to maintain optimum growth in broiler chickens raised under temperate or tropical conditions. However, Deyhim and Teeter (1993) who fed broilers with withdrawal VIT premix from 28 to 49 days of age and exposed them to cyclic ambient temperatures (24 °C to 35 °C), observed reduced weight gain, increased mortality and compromised FCR in broilers deprived of the premixes. The adverse effects of VIT and TRM premixes withdrawal on the growth performance of broilers were also described by Patel et al. (1997). The authors suggested that the lack of animal-based protein, which contains twice as much riboflavin and other B vitamins compared to soybean meal may be the reason for the depressed growth performance observed. However, in the present study, our diets also contained only maize and soybean meal as protein sources. Hence, there is no clear explanation as to these discrepancies, but the duration of withdrawal, strain of broilers used, and rearing system may have accounted for the inconsistencies.

As expected, male birds had higher BW ($p < .001$) and FI ($p < .001$) than their female counterparts (Table 2). Mortality rate was not affected by diet (Table 2). The relatively higher mortality rates noted in this study could be attributed to the hot and humid environment, as the birds were raised in a naturally-ventilated house. The detrimental effect of heat stress on broiler chickens particularly at market age is well established (Vale et al. 2010).

Table 4. Mean (±SEM) robusticity index where diet × gender interaction was significant at 42 days of age.

Diet	Male	Female
W3	$4.70^{y} \pm 0.0682$	$5.05^{ax} \pm 0.0852$
W2	$4.66^{y} \pm 0.0847$	$5.10^{ax} \pm 0.0847$
W1	4.70 ± 0.0584	$4.81^{b} \pm 0.0407$
Control	4.66 ± 0.0545	$4.68^{b} \pm 0.0791$

[a,b]Means bearing uncommon superscripts within a column differ significantly at $p < .05$.
[x,y]Means bearing uncommon superscripts within a row differ significantly at $p < .05$.
W3: VIT and TRM premixes withdrawal from 21 to 42 days; W2: VIT and TRM premixes withdrawal from 28 to 42 days; W1: VIT and TRM premixes withdrawal from 35 to 42 days; Control: No VIT and TRM premixes withdrawal.

Tibia bone strength

Diet × gender interaction was noted for RI ($p = .013$) as shown in Table 3. Diet affected RI among female birds but not among male birds (Table 4). The RI of W2 and W3 females were higher (less desirable) than those of the control and W1 ($p < .05$). Moreover, female birds had lower TBS than males ($p = .005$) (Table 3). Subjecting birds to W2 and W3 resulted in lower TBS than those fed on the control diet ($p = .001$). These findings indicate that the removal of VIT and TRM premixes from broiler diet for more than one week during the finisher stage was detrimental to TBS and RI of both male and female broilers. Similarly, Ebrahimnezhad et al. (2011) showed that TRM premix removal from 21 to 42 days adversely

affected bone strength in broilers. Rath et al. (2000) has reported that higher a concentration of Cu and Fe in the diet may stimulate bone growth and increase bone strength. Withdrawal of VIT premix from 29 to 42 days of age has also been reported to affect bone parameters adversely (Moravej et al. 2012a). Vitamin D, B6, C, and K are known to be essential for bone health as they are involved in the synthesis of bone matrix constituents (Weber 1999; Shim et al. 2012). However, despite the importance of VIT and TRM premixes for bone strength, Skinner et al. (1992) reported that withdrawal of both premixes from broiler finisher diets from 28 to 49 days had negligible effect on incidence of leg disorders. It appears that although bone strength could be affected by withdrawal of VIT and TRM premixes, it may not be severe enough to result in leg disorders in broilers.

It is interesting to note that male birds had better TBS and lower RI than females in this study. The lower RI of male birds suggested stronger and healthier bone structure (Reisenfeld 1972; Yalcin et al. 2001). Size and hormonal differences may account for the differences in bone strength (Rath et al. 2000). Faster-growing male broilers are characterised by higher serum growth hormone (GH) and testosterone levels (Morpurgo & Porter 1995; Kuhn et al. 1996). Both GH and testosterone affect bone formation via facilitating cortical and cancellous bone growth (Brook 1995; Rath et al. 1996). Therefore, the gender differences in androgen level and somatotrophic functioning may account for the inferior RI in female broilers deprived from VIT and TRM premixes for durations more than one week.

Incidence of footpad dermatitis

Neither diet nor gender ($p = .466$ and $p = .101$, respectively) affected the incidence of FPD in our study (Table 3). Similarly, Waldroup et al. (1968) reported no incidence of toe and hock dermatitis in broilers fed on a maize-soy diet without TRM premix. Although some previous studies have emphasised the importance of biotin deficiency in FPD incidence (Patrick et al. 1943; Jensen & Martinson 1969), our results did not confirm such findings. It is possible that that the 1–3-week VIT and TRM premixes withdrawal in our study was not a chronic deficiency to affect FPD incidence.

Immune response

Vitamins and trace minerals such as carotenoid, vitamin E, vitamin C, selenium and zinc showed to enhance immunity by maintaining the functional and structural integrity of important immune cells (Chew 1995; Muir et al. 2002; Singh et al. 2006; Lagana et al. 2007). On the contrary, our results indicated that irrespective of duration, withdrawal of VIT and TRM premixes did not influence antibody response against ND vaccinations when compared to controls (Table 3). Similarly, Deyhim and Teeter (1993) demonstrated negligible changes in antibody response in broilers deprived of vitamin premix and raised under either thermoneutral or heat stress conditions. Given the lack of significant differences in ND antibody responses between the control and the other groups, there appears to be no obvious explanation for the apparent higher values attained by the W1 birds when compared to their W2 and W3 counterparts.

Our results showed that male birds had lower antibody production than their female counterparts ($p = .003$) (Table 3). There are conflicting reports about the effect of gender on immune response, while some reports have demonstrated similar antibody response for both genders (Klingensmith et al. 1983; van der Zijpp et al. 1986), others showed superior response in females (Leitner et al. 1989). In this study, we noted that the females had a higher antibody response against ND vaccinations. This observation could be attributed to gender effects such as sex hormones or growth rate. Although testosterone may influence humoral and cell-mediated immune responses (Chen et al. 2009), it may not be a relevant explanation in broiler chickens (Shore et al. 1993). However, growth rate differences have been correlated with antibody response (Dunnington et al. 1993; Parmentier et al. 1996). It seems that the higher growth rate of male chickens comes, at least partially, at the cost of their humoral immune response.

Conclusions

Our results suggested that VIT and TRM premixes can be removed from 21 to 42 days of age without any adverse effects on growth performance, mortality rate, antibody response against ND vaccination or incidence of FPD in male and female broilers. However, withdrawal of both premixes for more than a week reduced bone-breaking strength.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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