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Abstract

An experiment was conducted to investigate the effect of rapeseed meal (RSM), sunflower meal (SFM), canola meal (CM) and guar meal (GM) respectively at 50, 60, 100 and 50g/kg in a maize soyabean based diet on growth performance, plasma mineral concentration, bone mineralisation and mineral digestibility in broiler chickens. The control diet had soya bean meal (SBM; 320g/kg). Day old Hubbard broilers were allocated to five treatments each with 4 replicates and 10 birds. Partial replacement of SBM with other protein sources resulted in heavier ($P<0.05$) birds compared to control. Bone mineralisation was not affected ($P>0.05$) by diets. Plasma Ca concentration was higher ($P<0.05$) in birds fed GM compared to all other treatments. Retention of P, Mg, Na, Cu was increased and Ca retention was decreased in birds fed control diet whereas, no differences ($P>0.05$) were observed between treatments in K, Fe and Mn retention values. In conclusion, alternative protein sources improved growth but had variable effects on mineral utilisation.

Introduction

The global increase in intensive poultry farming and constant increases in the cost of many conventional feed ingredients has urged a need to explore the use of non conventional feed stuff. It has been reported that the three main criteria's which determines the use of alternative feed stuff in poultry feed are its availability in economic quantities, its competitive price and most importantly understanding of its nutritive value (Ravindran and Blair. 1992). The use of these alternative feed resources, especially in very young birds, is generally limited due to their relatively higher proportion of antinutritive factors which could reduce nutrient utilization.

Although soyabean meal is the major protein source for the world's poultry production, other oil seeds such as canola seed, rapeseed, sunflower, guar meal are frequently used as alternative (Fenwick and Curtis, 1980; Khattak *et al,* 2004; Senkoylu and Dick, 1999; Hussan *et al,* 2012). In countries where soyabean meal has to be imported, its partial or complete replacement by locally available meal alternatives will lead to cost effective feed formulations. Anti-nutritive effects of inclusion of soyabean meal (SBM), rapeseed meal (RSM), canola meal (CM), cotton seed meal (CSM), guar meal (GM) and sunflower meal (SFM) in poultry ration has been well reviewed (Bell, 1993, Senkoylu and Dale, 1999 and Leeson and Summers, 2001).

The newest area to receive attention, from an environmental point of view, is mineral nutrition and excretion. Feeding minerals with higher availability to meet the specific requirements of the poultry can reduce the amount of the mineral excreted and thereby the environmental pollution is reduced (Pierce, *et al*, 2005). Minerals are essential for maintenance and normal functioning of the poultry therefore their sufficient supply should be ensured through feed because their deficiency can lead to reduced growth whereas over supply can harm production efficiency and will result in negative impact on the environment. Limited work has been done to explore the mineral profile of alternative protein sources. The objectives of the study are to evaluate the effects of diets containing RSM, SFM, CM and GM on growth performance, blood plasma and bone mineral concentration, dry matter digestibility and retention of minerals in broilers.

Materials and Methods

Two hundred day old male broiler chicks (Hubbard) were allocated randomly to 5 dietary treatments. Each treatment had 4 replicates with 10 chicks per cage A isonitrogenous

and isoenergetic maize and soyabean meal-based diet was used as a control treatment (A). The other 4 treatments were generated by replacing soya bean meal (SBM) with RSM, SFM, CM, and GM and were designated as treatment B, C, D and E respectively (Table 1). Birds were reared in 20 floor pens during the first 10 days and were fed on control diet A. On day 10, birds maintained within the same group were shifted to metabolic cages and were fed experimental diets. Feed and water was offered *ad-libitum*. Birds and feed were weighed at day 0, 10 and 21. Feed to gain ratio was determined using average feed intake and body weight gain values obtained during day 10 to 21.

Table 1. Calculated feed composition of experimental diets.

Ingredients (%)	**Experimental Treatments**				
	A	**B**	**C**	**D**	**E**
Maize	64.17	62.87	61.48	61.37	63.50
Soyabean Meal	32	28.37	28.69	24.98	27.62
Sunflower meal	-	-	6	-	-
Canola Meal	-	-	-	10	-
Rapeseed Meal	-	5	-	-	-
Guar meal	-	-	-	-	5
DCP	1.86	1.82	1.8	1.77	1.87
NaCl	0.25	0.25	0.25	0.25	0.25
Lime stone	1.37	1.34	1.41	1.31	1.35
DL Methionine	0.17	0.17	0.17	0.15	0.18
L Lysine	0.04	0.04	0.06	0.03	0.09
Soda bicarbonate	0.14	0.14	0.14	0.14	0.14
Calculated nutrient composition					
ME (Kcal/kg)	2900	2900	2900	2900	2900
Protein	19	19	19	19	19
Ether Extract	2.71	3.08	2.76	2.73	2.83
Fiber	3.29	3.75	3.85	4.29	3.16
Determined mineral concentration (mg/dl)					
Calcium	401	454	510	252	399
Phosphorous	5	2.7	4	2.2	5.6
Magnesium	256	255	245	215	258
Potassium	678	708	681	634	681
Sodium	157	162	161	135	152

At day 21, two birds per replicate cage were randomly selected for blood collection. Birds were euthanized and blood samples taken from wing vein were collected in heparin tubes. Plasma was separated by centrifugation of blood for 15 minutes at 3000 revolutions per minute (rpm) in centrifuge machine. Plasma was diluted with 10% trichloroacetic acid (TCA) before analyzing it on atomic absorption spectrophotometer and spectrophotometer (AOAC, 1990). After blood collection same birds were used for tibia collection. Left tibia was removed and placed in boiling water for 10 minutes in a glass beaker to remove muscles and connective tissues. The clean bone was dried at 105°C for 48 h in an oven. The dried tibia was ashed at 600 °C for 16 hours in labeled crucibles using an electric furnace. Tibia ash (0.025 g) was taken in a 50 ml flask & 0.2ml 5N HCl was added to dissolve ash. The mixture was shaken for 5 minutes and deionized water was added to make the volume up to 50 ml. This solution was used to quantify minerals by using atomic-absorption spectrophotometer (Perkin Elmer AA 400) except Phosphorous (P), which was measured using spectrophotometer (AOAC, 1990).

Total excreta collection method was used to determine dry matter digestibility (DMD) and mineral retention values. The feed and total excreta collected per cage over last 3 days were weighed. Excreta samples were dried in an oven at 105°C for 48hrs. The 5g of dried excreta was than ashed at 650 °C for 12 hours in crucibles in an electric furnace. 0.5g of ashed sample was taken in a flask and 10 ml of nitric acid was added and the flask was kept in hot water bath at 65°C for 15 minutes. 5 ml of perchloric acid was added to the flask and was kept again in hot water bath at 75°C for 15 mints. Then sample was dried on hot plate till 0.5 ml of the fluid was left. After filtration the sample was raised to 50 ml solution by repeated washing. This solution was used to determine mineral concentration.

The data obtained was subjected to statistical analysis using one way Analysis of Variance Technique using GenStat 14 statistical software package (IACR, Rothamstead, Hertfordshire, UK). All statements of significance are based on the probability level of $P<0.05$. Duncan multiple range test (DMR) was used to separate means.

Results and discussion

All birds fed diets containing alternative protein sources were heavier ($P<0.05$) and resulted in improved weight gain compared to control (Table 2). Feed intake was similar between treatments ($P>0.05$). Feed to gain ratios was numerically lower for birds fed SFM however, no statistical differences ($P>0.05$) were observed when compared between treatments. GM has been reported to have no adverse effects on body weights when incorporated up to 50 g/kg in poultry feed (Gutierrez *et al*., 2007). In contrast, Ahmed *et al.,* (2007) reported that body weights in broiler chicken were significantly reduced when CM (300 g/kg) was added in diets during 1-21d. These differences in growth response are probably due to differences in the variety and inclusion level of CM used in these studies.

Table 2. Growth performance (mean values ± SD) of birds fed different experimental diets during day 10-21.

Treatments	Final body weight (g/bird/day)	Weight gain (g/bird/day)	Feed Intake (g/bird/day)	Feed:gain
A (Control)	513^{a}± 0.03	278^{a}±0.03	768^{a}±0.01	2.5^{a}±0.15
B (RSM)	560^{b}±0.03	324^{b}±0.03	783^{a}±0.03	2.1^{a}±0.49
C (SFM)	639^{c}±0.03	403^{c}±0.03	825^{a}±0.03	1.8^{a}±0.18
D (CM)	621^{c}±0.01	385^{bc}±0.01	884^{a}±0.13	2.1^{a}±0.77
E (GM)	546^{b}±0.05	310^{b}±0.05	863^{a}±0.03	2.8^{a}±0.55

The mean in the same column having different superscript are significantly different at $P<0.05$

Bone mineralization was not affected ($P>0.05$) by diets (Table 3). Ca concentration in plasma was higher for birds fed GM compared to all other treatments (Table 4). It is important to know that there is hormonal regulation to control homeostasis in the extracellular fluid. Therefore, differences in blood levels only occur when the regulatory mechanism is no longer able to maintain the extracellular content of the mineral within the narrow physiological range (Van der Vede *et al.* 1986). However, it was not clear in the present study what triggered the plasma Ca level to elevate.

Table 3. Mineral concentration (mg/100g, mean ± SD) in bone of birds fed different experimental diets at day 21.

Treatment	Ca	P	Mg	K	Fe
A (Control)	1003[a]±186	8.4 [a]±0.8	88.2 [a]±8.7	60.0 [a]±15.3	55.7 [a]±15.4
B (RSM)	859.5 [a]±197	8.6 [a] ±1.2	80.2 [a]±19.1	67.5 [a]±25.0	66.5 [a]±30.8
C (SFM)	919.8 [a]±147	9.7 [a]±1.8	86.2 [a]±13.7	60.7 [a]±4.7	58.7 [a]±21.0
D (CM)	916.0 [a]±149	10.0 [a]±1.6	87.5 [a]±17.9	63.0 [a]±5.7	50.5 [a]±9.6
E (GM)	908.8 [a]±94	11.6 [a]±6.1	72.5 [a]±7.3	60.0 [a]±8.4	56.2 [a]±11.3

The mean in the same column having different superscript are significantly different at P<0.05. Where Ca = Calcium, P = Phosphorous; Mg = Magnesium; k = Potassium; Fe = Ferrous

Table 4. Mineral concentration (mg/dl, mean ± SD) in plasma of birds fed different experimental diets at day 21.

Treatment	**Ca**	**Mg**	**P**
A (Control)	9.0[a]±2.36	3.0[a]±0.44	12.6[a]±6.59
B (RSM)	9.1[a]±1.54	3.2[a]±0.27	7.9[a]±1.07
C (SFM)	8.8[a]±1.72	3.2[a]±0.23	13.2[a]±4.91
D (CM)	9.2[a]±2.36	3.1[a]±0.26	12.3[a]±8.38
E (GM)	11.8[b]±1.12	3.4[a]±0.26	8.8[a]±3.03

The mean in the same column having different superscript are significantly different at P<0.05. Where Ca = Calcium, P = Phosphorous; Mg = Magnesium.

Percent dry matter digestibilities (DMD) and mineral retention values of birds fed different experimental diets are presented in Table 5. It was interesting to note that alternative protein sources had varying effects on DMD and major mineral retention. Trace minerals (Cu, Fe, Mn) were not affected by the diet. As expected birds fed control diets had significantly higher DMD and mineral retention except in Ca where the retention values were lowest (P<0.05). The improvement in digestibility and retention values is clearly due to increased quantity of soyabean meal in control diet as the amino acids in soybean meal are known to be highly digestible (Britzman 1998).

Table 5. Percent dry matter digestibility (DMD) and mineral retention (mean values ±SD) of birds fed experimental diets

Treatments	**DMD**	**Ca**	**P**	**Mg**	**K**	**Na**	**Cu**	**Fe**	**Mn**
A (Control)	81.6[a] ±4.8	45.9[a] ±8.9	91.2[a] ±6.9	83.6[a] ±3.89	95.3[b] ±1.2	87.7[a] ±0.8	64.5[a] ±7.3	69.6[a] ±6.9	73.8[a] ±12.3
B (RSM)	62.2[b] ±1.7	88.7[b] ±9.7	79.5[a] ±7.6	56.2[b] ±3.86	89.3[b] ±3.7	57.5[b] ±2.5	33.8[b] ±3.8	66.5[a] ±15.8	46.1[a] ±30.6
C (SFM)	76.2[c] ±2.4	95.6[b] ±6.3	51.6[b] ±5.3	68.7[b] ±3.24	91.7[b] ±0.1	64.9[c] ±2.0	28.9[b] ±2.6	68.8[a] ±8.9	48.7[a] ±30.5
D (CM)	74.1[c] ±2.5	92.1[b] ±3.7	32.7[c] ±5.8	62.3[b] ±3.90	88.3[b] ±3.2	64.0[c] ±2.0	30.6[b] ±6.6	63.7[a] ±7.0	39.4[a] ±12.7
E (GM)	73.0[c] ±1.5	96.6[b] ±3.2	65.4[b] ±5.1	66.0[b] ±3.24	86.8[b] ±2.5	53.2[b] ±3.9	34.3[b] ±2.2	23.0[a] ±11.4	80.2[a] ±10.6

In conclusions it can be suggested RSM, SFM, CM and GM has a potential to partially replace soyabean meal in poultry diets without any detrimental effect on the birds performance. The fact that dietary composition altered DMD and mineral retention values further suggests that it is important that alternative protein ingredients should be analysed for mineral concentration and than those values should be used to formulate feeds to ensure that the minerals are provided in sufficient quantities to achieve not only optimal health, welfare and growth but also to minimise the excretion of minerals into the environment.

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