Interactive Effects of Phytase Supplementation and Dietary Oil Source on Humoral Immune Response, Leukocyte Profile, Mineral Digestibility, and Growth Performance in Broiler Chickens

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Abstract

Introduction:

This study evaluated the interactive effects of phytase supplementation and dietary oil source on growth performance, calcium and phosphorus digestibility, bone mineralization, and immune-related responses in broiler chickens.

Material and methods:

A total of 540 male Ross 308 broiler chickens were allocated to six dietary treatments in a completely randomized 2 × 3 factorial arrangement with two phytase levels (0 and 500 FTU/kg) and three oil sources (soybean oil, soybean–palm oil blend, and palm oil), with six replicates per treatment over a 42-day period.

Results:

Significant phytase × oil source interactions were detected for body weight gain ($P < 0.05$), feed intake ($P < 0.05$), mortality rate ($P < 0.05$), and ileal calcium and phosphorus digestibility ($P < 0.01$). Broilers fed the soybean oil diet supplemented with phytase achieved the highest body weight gain and feed intake among all treatments. Ileal calcium digestibility was highest with the non-supplemented soybean–palm oil blend, while phosphorus digestibility was highest with the phytase-supplemented blend and non-supplemented palm oil. Phytase supplementation increased serum calcium concentration ($P < 0.05$), but neither phytase nor oil source, nor their interaction, significantly affected serum phosphorus, tibia mineral characteristics (calcium, phosphorus, and total ash), or immune response parameters, including antibody titers against sheep red blood cells and leukocyte profiles ($P > 0.05$).

Conclusion:

In conclusion, the nutritional efficacy of phytase on growth performance and ileal mineral digestibility is strongly modulated by the dietary lipid source in broiler chickens. These findings indicate that combining phytase supplementation with a partial or complete replacement of soybean oil with palm oil offers an effective and economically viable feeding strategy without compromising bone traits or systemic immunity.

Keywords: Broiler chicken, immune response, mineral digestibility, palm oil, phytase, soybean oil

1. Introduction

Efficient poultry nutrition is essential for sustainable poultry production because feed accounts for approximately 65–75% of the total production cost of meat and eggs (1).

Dietary lipids are widely incorporated into broiler diets to increase dietary energy density, as they provide approximately 2.25 times more metabolizable energy than carbohydrates due to their highly reduced carbon structure (1, 2). Beyond their contribution to energy supply, dietary fats improve growth performance and feed efficiency, enhance the absorption of fat-soluble vitamins, increase feed palatability, reduce feed dust, and prolong digesta retention time, thereby promoting nutrient utilization (1, 2). The nutritional value of dietary lipids, however, is influenced not only by their energy content but also by their fatty acid composition, which can affect nutrient digestion, mineral bioavailability, oxidative status, and physiological responses in poultry (1, 3-5).

Despite their numerous nutritional benefits, dietary lipids pose a challenge through their interactions with other dietary constituents. Previous studies have demonstrated that dietary lipid composition can influence the bioavailability of minerals through the formation of insoluble calcium–fatty acid soaps in the intestinal lumen. This process, which is more pronounced with saturated fatty acids, reduces mineral absorption and skeletal mineralization, resulting in lower calcium and bone ash contents in poultry (3, 6-8).

On the other hand, the excessive dietary polyunsaturated fatty acids may enhance lipid peroxidation, alter antioxidant status, and influence inflammatory and immune-related physiological responses in poultry (4, 5). Furthermore, an appropriate combination of saturated and unsaturated fatty acids may improve fat digestibility compared with using either type of fat alone (1, 9). Among dietary lipid sources, palm oil has attracted attention due to its relatively balanced composition of saturated and unsaturated fatty acids, which may improve lipid utilization and nutritional value. In addition, its relatively lower price than many other vegetable oils makes it an economically attractive ingredient for poultry feed formulations. Moreover, its bioactive

antioxidant compounds, including tocopherols and tocotrienols, can reduce lipid peroxidation and oxidative stress, thereby supporting immune function in poultry (10-12).

Additionally, phytate, the principal storage form of phosphorus in plant-derived feed ingredients, forms complexes with minerals and proteins, reducing their absorption (13). Phytase supplementation has become an effective nutritional strategy for improving phosphorus utilization through enzymatic hydrolysis of phytate, thereby releasing phytate-bound minerals and enhancing calcium and phosphorus availability and nutrient digestibility in broilers (14-16).

Although dietary lipid sources and phytase supplementation have individually been shown to influence nutrient utilization and physiological responses in broilers, their interactive effects remain insufficiently understood. Moreover, the combined use of palm oil and phytase has been reported to enhance nutrient digestibility and metabolizable energy in other animal species (17, 18). However, despite the well-documented individual effects of dietary oil sources and phytase in broiler nutrition, limited information is available regarding their interactive effects on ileal calcium and phosphorus digestibility and immune-related physiological responses, particularly in diets containing palm oil and soybean oil.

Hence, the aim of this study was to investigate the effect of using phytase in diets containing palm oil and soybean oil on ileal calcium and phosphorus digestibility, mineral utilization, and immune-related physiological parameters in broiler chickens.

2. Materials and methods

In this study, a total of 540 one-day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery in Iran. Upon arrival, chicks were individually weighed and randomly

allocated to six dietary treatments with six replicates per treatment and 15 birds per replicate. The experiment was arranged in a completely randomized design with a 2×3 factorial arrangement, consisting of two levels of phytase (0 and 500 FTU/kg) and three levels of soybean oil replacement with palm oil (0, 50, and 100%).

The dietary treatments were as follows:

T1: 3% soybean oil (without phytase)

T2: 3% soybean oil + phytase

T3: 1.5% soybean oil + 1.5% palm oil (without phytase)

T4: 1.5% soybean oil + 1.5% palm oil + phytase

T5: 3% palm oil (without phytase)

T6: 3% palm oil + phytase

Phytase (Bonfezyme®, 10,000 FTU/g; Bon Da Faravar Development Company, Iran) was included to provide 500 FTU/kg in the diet.

2.1. Diets and management

The experimental diets were formulated to meet the nutrient requirements of Ross 308 broiler chickens based on breeder recommendations and NRC (1994) guidelines. Diets were formulated to be isocaloric and isonitrogenous within each feeding phase. Birds were reared for six weeks and vaccinated according to the following schedule using vaccines produced by the Razi Vaccine and Serum Research Institute (Iran). On day 1 of age, chicks received H120 and B1 vaccines against infectious bronchitis (IB) and Newcastle disease (ND), respectively, by spray administration (1.2 doses per bird). At 7 days of age, birds received a combined avian influenza (AI) and ND vaccine

subcutaneously in the back of the neck (one dose per bird), followed by a clone ND vaccine administered via eye drop (1.2 doses per bird) .An infectious bursal disease (IBD; Gumboro) vaccine was administered orally at 15 and 22 days of age (1.2 doses per bird). At 18 days of age, the clone ND vaccine was administered orally (1.2 doses per bird). Finally, a heat-resistant ND vaccine was administered orally at 28 and 35 days of age (1.2 doses per bird). Feed and water were provided ad libitum throughout the experimental period. Broilers were fed starter, grower, and finisher diets during days 1–10, 11–24, and 25–42, respectively (**Table 1**) .Room temperature was initially maintained at 29°C and gradually reduced by 1°C every 3 days until reaching 21°C.

2.2. Serum calcium and phosphorus analysis

At days 10, 24, and 42, blood samples were collected from three birds per replicate into plain vacutainer tubes (without anticoagulant). The blood samples were allowed to clot and were then centrifuged at 3,000 rpm for 10 min. The resulting serum was separated and stored at −80°C until further analysis. Serum calcium and phosphorus concentrations were determined using commercial DIALAB® kits with an automated analyzer (BT 1500).

2.3. Ileal digestibility

Chromium oxide (0.3%) was added to diets during the last week of the experiment as an indigestible marker for determination of ileal calcium and phosphorus digestibility (19). On day 42, three birds per replicate were randomly selected and slaughtered by cervical dislocation. The abdominal cavity was immediately opened, and the ileal segment between Meckel's diverticulum and 2 cm anterior to the ileocecal junction was excised. Ileal digesta samples were collected from

each replicate into clean, sterile plastic containers and immediately stored at −20°C until further analysis.

Calcium and phosphorus concentrations in feed and ileal digesta samples were determined using the Fenton spectrophotometric method (20). Samples were first ashed at 550°C in a muffle furnace. The resulting ash was mixed with a digestion solution (10 g sodium molybdate dihydrate dissolved in 500 mL of a mixture containing distilled water, concentrated sulfuric acid, and 70% perchloric acid at a ratio of 150:150:200, v/v/v) and heated on a hot plate (surface temperature up to 300°C) until complete color development was achieved. Samples were further heated for 10 to 15 minutes, removed from the hot plate, and allowed to cool. Digested samples were quantitatively transferred to 200 mL volumetric flasks and diluted with distilled water. Optical density (OD) was measured at 440 nm using a spectrophotometer (Unicam 8620 UV/vis).

Apparent ileal digestibility of calcium and phosphorus was calculated using chromium oxide as an indigestible marker according to the following equation (21):

$$Y = 100–[100 \times \frac{Cr_2O_3\ diet \times M\ digesta}{Cr_2O_3\ digesta \times M\ diet}]$$

where Y represents nutrient digestibility (%); Cr_2O_3 represents the chromium oxide concentration in the diet or ileal digesta; and M represents the nutrient concentration in the diet or ileal digesta.

2.4. Tibia mineralization

To determine bone mineral content, left tibiae from slaughtered birds were collected and immediately frozen at –20°C until analysis. Samples were cleaned of soft tissues, dried at 105°C,

defatted with ethyl ether extraction, and ashed at 600°C to determine ash, phosphorus, and calcium according to AOAC (1990).

2.5. Immune response

Humoral immunity was assessed using the sheep red blood cell (SRBC) assay. On day 23, 1 mL of 10% SRBC suspension was intravenously injected into two birds per replicate. Blood samples were collected 7 and 14 days after immunization for determination of antibody titers and leukocyte profiles. Heterophils and lymphocyte percentages were determined according to Gross and Siegel (1983) (22). Serum was separated by centrifugation at 3,000 rpm for 10 min, and total anti-SRBC antibody titers were determined using the hemagglutination (HA) method (23). All antibody titer data were expressed as $\log_2$ values.

2.6. Statistical analysis

Data were analyzed using a completely randomized design with a with a 2×3 factorial arrangement to evaluate the effects of phytase supplementation (0 and 500 FTU/kg) and oil sources (soybean, palm, and their mixture). Normality of data was assessed using SAS software (version 9.3; SAS Institute Inc., Cary, NC, USA), and analysis was performed using the GLM procedure with the following model:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + e_{ij}$$

where $Y_{(ij)}$ = dependent variable; μ = overall mean; A_i = oil level; B_j = phytase level; AB_{ij} = interaction effect of phytase and oil; and e_{ij} = random error associated with each observation. Treatment means were compared using Tukey's multiple range test, and differences were considered significant at $P < 0.05$.

Table 1. Range of feed ingredient inclusion levels and calculated nutrient composition of the six experimental diets during the starter (d 1–10), grower (d 11–24), and finisher (d 25–42) periods.

Ingredients (%)	**Dietary treatments from 1 to 42 days**					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Corn	53.3-59.16	51.65-58.38	53.22-59.48	51.96–59.02	53.42–59.81	52.27–59.42
Soybean meal 44%	28.63-35.56	31.99-36.36	27.68-34.99	32.32–36.29	26.73–34.42	32.01–36
Corn gluten	3-3.5	0-2.07	3.59-3.85	0–2.12	4.18–4.21	0.16–2.17
Soybean oil	3-5	3-5	1.5-2.5	1.5–2.5	0	0
Palm oil	0	0	1.5-2.5	1.5–2.5	3–5	3–5
Wheat bran	0	0-3.09	0	0–2.73	0	0–2.36
Dicalcium phosphate	1.76-2.10	0.77-1.11	1.77-2.11	0.78–1.12	1.78–2.12	0.79–1.12
Limestone	0.89-1.04	1.16-1.65	0.89–1.04	1.32–1.4	0.89–1.04	1.15–1.31
Vitamin–mineral premix *	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Bicarbonate	0.1	0.1	0.1	0.1	0.1	0.1
L-lysine	0.3-0.39	0.19-0.36	0.32–0.41	0.19–0.36	0.34–0.42	0.2–0.36
DL-methionine	0.29-0.35	0.3-0.35	0.28–0.34	0.3–0.35	0.28–0.34	0.3–0.35
L-threonine	0.09-0.13	0.07-0.14	0.09–0.14	0.07–0.13	0.09–0.14	0.07–0.13
Phytase	0	0.005	0	0.005	0	0.005
Calculated composition						
	Starter (1–10 d)		**Grower (11–24 d)**		**Finisher (25–42 d)**	
ME (kcal/kg)	3000		3100		3200	
Crude protein (%)	23		21.5		20	
Calcium (%)	0.96		0.87		0.81	
Available P (%)	0.48		0.44		0.41	
Methionine + cysteine (%)	1.08		0.99		0.94	
Lysine (%)	1.44		1.29		1.19	

Values are presented as ranges representing the minimum and maximum inclusion levels across the starter (d 1–10), grower (d 11–24), and finisher (d 25–42) periods.

Phytase was supplemented at 500 FTU/kg in T2, T4, and T6; T1, T3, and T5 received no phytase.

*Vitamin–mineral premix provided per kg of complete diet: vitamin A, 10,000–13,000 IU; vitamin D_3, 4,000–5,000 IU; vitamin E, 55–80 IU; vitamin K_3, 3.2–4.0 mg; thiamine, 3–5 mg; riboflavin, 7–9 mg; nicotinic acid, 50–70 mg; calcium pantothenate, 15–

25 mg; pyridoxine, 3–5 mg; biotin, 0.22–0.35 mg; folic acid, 1.8–2.5 mg; vitamin B_{12}, 0.016–0.020 mg; Cu, 16 mg; Zn, 120 mg; Mn, 120 mg; Fe, 20 mg; Se, 0.30 mg; and I, 1.25 mg.

3. Results

3.1. Growth performance and mortality

The effects of dietary treatments on growth performance are shown in **Table 2**. Phytase supplementation significantly increased body weight gain (BWG) and feed intake (FI) by 21.13 and 32.18 grams, respectively ($P < 0.05$), whereas feed conversion ratio (FCR) was not affected ($P > 0.05$). Oil source influenced BWG and FCR ($P < 0.05$), with birds fed soybean oil exhibiting greater BWG than those fed palm oil or the soybean–palm oil blend, whereas FI was not affected by oil source ($P > 0.05$). A significant interaction between phytase supplementation and oil source was observed for BWG and FI ($P < 0.05$), but not for FCR ($P > 0.05$). Broilers fed the soybean oil diet supplemented with phytase showed higher BWG than those receiving the other dietary treatments ($P < 0.05$). Likewise, FI was higher in birds fed the soybean oil diet supplemented with phytase than in those fed the soybean oil diet without phytase ($P < 0.05$), while the remaining treatments did not differ significantly.

Table 2 - Effect of phytase supplementation and different oil sources on performance trait of broiler chickens fed the experimental diets from d 1 to d 42.

Treatment		Performance traits		
Phytase (FTU/kg)	Oil sources	Body weight (g)	Feed intake (g)	FCR (g/g)
0	Soybean	809.78[b]	1301.90[b]	1.44
0	Soybean + palm	804.71[b]	1322.90[ab]	1.49
0	Palm	801.63[b]	1322.95[ab]	1.47
500	Soybean	845.95[a]	1378.31[a]	1.46

500	Soybean + palm	811.21[b]	1345.54[ab]	1.51
500	Palm	822.36[b]	1330.45[ab]	1.45
Pooled SEM		5.36	13.74	0.01
Main effects				
Phytase	0	805.38[b]	1319.25[b]	1.472
	500	826.51[a]	1351.43[a]	1.478
Oil	Soybean	827.86[a]	1340.10	1.45[b]
	Soybean + palm	807.96[b]	1339.22	1.50[a]
	Palm	812[b]	1326.70	1.46[b]
***P*-values**				
Phytase		<.0.01	<.0.01	0.48
Oil		<.0.01	0.55	<.0.01
Phytase × Oil		<.0.05	<.0.05	0.08

[a,b,c] Means with different superscripts in the same row differ significantly (P < 0.05).

SEM: standard error of the mean.

Mortality rate throughout the experimental period is presented in **Table 3.** A significant phytase × oil source interaction was observed for mortality rate (P < 0.05). Broilers fed the soybean–palm oil diet supplemented with phytase and those fed the palm oil diet without phytase had the lowest mortality (0%), which was significantly lower than that of birds fed the soybean oil diets and the phytase-supplemented palm oil diet (P < 0.05), but did not differ from birds fed the soybean–palm oil (P > 0.05).

Table 3 – Effect of phytase supplementation and dietary oil sources on mortality rate of broiler chickens during the experimental period.

Treatment	Soybean oil without phytase	Soybean oil with phytase	Soybean + palm oil without phytase	Soybean + palm oil with phytase	Palm oil without phytase	Palm oil with phytase
Mortality (%)	7.78[a]	12.22[a]	6.67[ab]	0[b]	0[b]	8.89[a]

Note: Means with different superscripts (a, b) differ significantly ($P < 0.05$).

3.2. Nutrient digestibility

Ileal digestibility values of calcium, and phosphorus are summarized in **Table 4.** Digestibility measurements were determined using chromium oxide as an indigestible marker. Phytase supplementation significantly increased the ileal digestibility of both calcium and phosphorus ($P < 0.05$). Oil source also affected the digestibility of both minerals ($P < 0.05$). Among the dietary oil sources, the soybean–palm oil blend resulted in the greatest calcium digestibility, followed by palm oil, whereas soybean oil showed the lowest calcium digestibility ($P < 0.05$). For phosphorus digestibility, palm oil and the soybean–palm oil blend resulted in greater phosphorus digestibility than soybean oil ($P < 0.05$).

Table 4– Effect of experimental treatments on ileal digestibility of calcium and phosphorus at the end of the experimental period.

Treatment		Ileal digestibility	
Phytase (FTU/kg)	Oil sources	Ca (%)	P (%)
0	Soybean	44.87[c]	44.20[d]
0	Soybean+ palm	60.66[a]	43.61[d]
0	Palm	54.16[b]	60.49[ab]

500	Soybean	56.72[b]	58.97[b]
500	Soybean+ palm	55.32[b]	64.09[a]
500	Palm	55.09[b]	48.74[c]
Pooled SEM		0.76	0.83
Main effects			
Phytase	0	53.23[b]	49.43[b]
	500	55.71[a]	57.27[a]
Oil	Soybean	50.79[c]	51.58[b]
	Soybean + palm	57.99[a]	53.85[a]
	Palm	54.62[b]	54.61[a]
***P*-values**			
Phytase		<.0.01	<.0.01
Oil		<.0.01	<.0.01
Phytase × Oil		<.0.01	<.0.01

[a,b,c,d] Means with different superscripts in the same column differ significantly (P < 0.05).

SEM: standard error of the mean.

A significant interaction between phytase supplementation and dietary oil source was observed for both calcium and phosphorus digestibility (P < 0.05). Broilers fed the soybean–palm oil diet without phytase exhibited greater calcium digestibility than those receiving the other dietary treatments, whereas birds fed the soybean oil diet without phytase had the lowest calcium digestibility (P < 0.05). For phosphorus digestibility, the highest value was noted in birds fed the phytase-supplemented diet with the soybean–palm oil blend and those fed the palm oil diet without phytase. The lowest phosphorus digestibility was observed in birds fed the soybean oil diet without phytase and those consuming the soybean-palm oil without phytase (P < 0.05).

3.3. Serum calcium and phosphorus

The overall mean serum calcium and phosphorus concentrations across the sampling period (days 10, 21, and 42) are presented in **Table 5**. Phytase supplementation significantly increased serum calcium concentration ($P < 0.05$), whereas serum phosphorus concentration was not affected ($P > 0.05$). Dietary oil source had no significant effect on serum calcium or phosphorus concentrations ($P > 0.05$). Furthermore, no significant interaction between phytase supplementation and dietary oil source was observed for either serum calcium or phosphorus concentrations ($P > 0.05$).

3.4. Tibial mineralization

Results regarding tibial mineral characteristics, including calcium, phosphorus, and ash contents, are summarized in **Table 5**. Neither phytase supplementation, dietary oil source, nor their interaction had a significant effect on tibial calcium, phosphorus, or ash content ($P > 0.05$).

Table 5. Effect of phytase supplementation and dietary oil sources on serum and tibial calcium and phosphorus concentrations in broiler chickens fed experimental diets from 1 to 42 days of age.

Treatment		Serum		Tibia bone		
Phytase (FTU/kg)	Oil sources	Ca (mg/dl)	P (mg/dl)	Ca (%)	P (%)	Total ash (%)
0	Soybean	6.03	4.93	13.27	6.26	37.39
0	Soybean+ palm	6.29	5.60	13.29	6.26	37.82
0	Palm	6.35	5.99	14.03	6.61	36.51
500	Soybean	6.05	5.48	15.25	6.56	39.61
500	Soybean+ palm	6.82	5.72	13.06	5.78	37.05
500	Palm	6.78	6.09	14.51	6.64	39.54
Pooled SEM		0.29	0.21	1.11	0.37	1.63

Main effects						
Phytase	0	6.22[b]	5.51	13.53	6.37	37.24
	500	6.78[a]	5.77	14.27	6.33	38.73
Oil	Soybean	6.56	6.04	14.26	6.41	38.50
	Soybean + palm	6.38	5.21	13.17	6.02	37.43
	Palm	6.55	5.66	14.27	6.62	38.02
***P*-values**						
Phytase		<0.05	0.14	0.43	0.87	0.28
Oil		0.78	0.06	0.54	0.30	0.80
Phytase × Oil		0.88	0.49	0.60	0.58	0.49

[a,b] Means with different superscripts in the same column differ significantly (P < 0.05).

SEM: standard error of the mean.

1.1. Immune responses

The effects of dietary treatments on immune response parameters measured at 30 and 37 days of age are presented in **Table 6.** No significant effects of phytase supplementation, dietary oil source, or their interaction were observed on anti-SRBC antibody titers, heterophil percentage, lymphocyte percentage, or the heterophil-to-lymphocyte (H/L) ratio at either sampling age (P > 0.05).

Table 6. Effects of phytase supplementation and dietary oil sources on immune response parameters in broiler chickens at 30 and 37 days of age.

Treatment		30 d of age				37 d of age			
Phytase (FTU/kg)	Oil sources	SRBC antibody	Heterophil (%)	Lymphocyte (%)	H/L*	SRBC antibody	Heterophil (%)	Lymphocyte (%)	H/L*

0	Soybean	5.25	20.33	79.66	0.25	5	20.27	80.83	0.24
0	Soybean+palm	5.63	23.70	76.30	0.33	4.75	17.83	82.16	0.22
0	Palm	5.08	18.20	82.40	0.23	4.41	20	80	0.26
500	Soybean	5.58	17.60	82.40	0.21	4.66	17.91	82.08	0.23
500	Soybean+ palm	5	28.85	71.14	0.49	4.33	17.66	82.33	0.23
500	Palm	5.83	37.25	52.75	0.69	4.25	21.33	78.66	0.31
Pooled SEM		0.24	4.49	4.49	0.09	0.24	2.89	2.90	0.06
Main effects									
Phytase	0	5.32	20.74	79.46	0.27	4.72	19.37	81.00	0.24
	500	5.47	27.90	72.10	0.47	4.42	18.97	81.03	0.26
Oil	Soybean	5.42	18.97	81.03	0.24	4.83	19.09	81.46	0.24
	Soybean+palm	5.32	26.28	73.72	0.42	4.54	17.75	82.25	0.23
	Palm	5.46	27.73	72.58	0.47	4.33	20.67	79.33	0.29

***P*-values**		SRBC antibody	Heterophil (%)	Lymphocyte (%)	H/L*
	Phytase	0.53	0.49	0.08	0.24
	Oil	0.36	0.57	0.14	0.32
	Phytase × Oil	0.06	0.16	0.07	0.15

* H/L: Heterophil-to-Lymphocyte ratio.

SEM: standard error of the mean.

2. Discussion

In the present study, phytase supplementation increased body weight gain and feed intake but did not significantly affect feed conversion ratio (FCR), possibly through phytate hydrolysis, which reduces its anti-nutritional effects and enhances phosphorus and nutrient availability (٢٤).

Previous studies have reported variable effects of phytase on broiler performance, with some showing no significant effects on body weight or feed intake (25) and others reporting improvements in body weight gain, feed intake, or feed efficiency (26-29). Similarly, the lack of an effect on FCR in the present study agrees with Das et al. (30) and Sampath et al. (31), although reductions in FCR have also been reported (٢٧, ٣٢-٣٤). Such variation may reflect differences in phytase source, dose and activity, dietary composition, bird age, and experimental conditions.

Dietary oil source also affected growth performance. Soybean oil resulted in greater body weight gain than palm oil or the soybean–palm oil blend, while feed intake was unaffected.

This response may be related to the higher proportion of unsaturated fatty acids in soybean oil, which are generally digested and absorbed more efficiently than saturated fatty acids (35). Previous studies have reported inconsistent effects of dietary oil sources on broiler performance, including weight gain, feed efficiency, and feed intake (30, 31, 36-41).

In contrast, the soybean-palm oil blend resulted in a higher FCR than either oil alone, which may reflect differences in fatty acid composition, lipid digestibility, and interactions between saturated and unsaturated fatty acids affecting fat utilization and energy availability (3, 35). Because most previous studies evaluated soybean and palm oils separately, direct comparisons with the present findings are limited.

The significant phytase × oil source interaction for body weight gain and feed intake, with the highest values observed in the soybean oil + phytase group, suggests that the growth response to phytase may depend on dietary lipid source, potentially reflecting the combined effects of phytate hydrolysis and fatty acid digestibility. The greater feed intake may have contributed to the higher body weight gain, although comparable interactions have rarely been reported.

Despite these improvements in body weight gain and feed intake, no significant phytase × oil interaction was observed for FCR, indicating that the increased feed intake was accompanied by a proportional increase in body weight gain rather than improved feed efficiency. However, increased weight gain can lead to chickens reaching slaughter weight earlier. This phenomenon helps to reduce the length of the rearing period and increase the number of rearing periods per year. Since all diets were isocaloric and isonitrogenous, the observed differences may be related primarily to lipid quality and nutrient utilization rather than dietary energy or protein levels.

The significant phytase × oil source interaction for mortality, with the lowest mortality observed in the phytase-supplemented soybean–palm blend and unsupplemented palm oil treatments, contrasts with previous reports showing no significant effects of dietary oil source or phytase on broiler mortality (37, 42-47). This finding should therefore be interpreted cautiously, given differences in diet composition, phytase level, genotype, and environmental conditions.

Phytase supplementation significantly improved apparent ileal calcium and phosphorus digestibility, consistent with previous reports (27, 48, 49). This effect is primarily attributed to phytate hydrolysis, which reduces phytate–mineral complex formation and releases phytate-bound phosphorus, thereby increasing calcium and phosphorus availability for intestinal absorption (50-

53) .The improvement in phosphorus digestibility is well established (27, 49, 53-55), whereas the response of calcium digestibility to phytase has been more variable. She et al. (49) reported increased calcium and phosphorus digestibility, while Amerah et al. (53) found no significant effect on calcium digestibility, and Van et al. (56) reported differential responses depending on dietary conditions. This variability may reflect the influence of dietary Ca balance and fatty acid interactions on calcium utilization, whereas phosphorus release is more directly linked to phytate hydrolysis.

Dietary oil source also affected mineral digestibility. The soybean–palm oil blend resulted in greater calcium digestibility than either oil alone, while palm oil and the blend increased phosphorus digestibility compared with soybean oil. These effects may reflect differences in fatty acid composition and fatty acid–mineral interactions, particularly calcium-soap formation (3, 8).

Zhong et al. (57) similarly reported increased calcium digestibility with flaxseed oil alone or combined with palm oil, supporting a potential role of fatty acid composition and saturation in mineral utilization. However, the mechanism underlying the greater calcium digestibility of the soybean–palm blend remains unclear and requires further investigation.

Phytase supplementation significantly increased serum calcium concentration, whereas serum phosphorus remained unchanged despite the improvement in ileal digestibility. As discussed above, the increase in serum calcium may reflect greater calcium availability following phytate hydrolysis (52).

In contrast, circulating phosphorus is tightly regulated by PTH, vitamin D, and FGF23; therefore, increased intestinal phosphorus availability does not necessarily increase serum phosphorus concentration because absorbed phosphorus is subject to skeletal, renal, and endocrine regulation.

329 This is consistent with previous reports showing improved phosphorus utilization without
330 corresponding increases in serum phosphorus following phytase supplementation (27, 58).

331 Dietary oil source and its interaction with phytase did not affect serum calcium or phosphorus,
332 suggesting that differences in ileal mineral digestibility were insufficient to alter circulating
333 mineral concentrations, likely due to tight mineral homeostasis (15, 59).

334 In the present study, neither phytase supplementation, dietary oil source, nor their interaction
335 significantly affected tibial calcium, phosphorus, or total ash content. Similar findings have been
336 reported when dietary mineral supply was adequate (48, 60-62), whereas positive responses to
337 phytase have also been observed (25, 26, 33, 63), suggesting that skeletal responses depend on
338 mineral availability, phytate content, phytase dose, and phosphorus limitation.

339 Dietary oil source may influence skeletal development through differences in fatty acid
340 composition and calcium utilization, with unsaturated fatty acids potentially favoring mineral
341 availability compared with highly saturated fatty acids.

342 Dietary fatty acid composition may also influence bone mineralization, with some studies
343 reporting more favorable bone characteristics with unsaturated than saturated fat sources (64, 65).
344 However, no such effect was observed among the oil sources evaluated in the present study

345 The absence of changes in tibial mineralization despite improved ileal calcium and phosphorus
346 digestibility may reflect mineral homeostasis, as greater intestinal mineral availability does not
347 necessarily increase bone deposition when skeletal mineral requirements are adequately met (66).

348 In the present study, the use of phytase and different oil sources did not significantly affect immune
349 response parameters, including SRBC antibody titers, heterophil percentage, lymphocyte
350 percentage, and heterophil-to-lymphocyte (H/L) ratio. The absence of significant changes suggests

that neither phytase supplementation nor dietary oil source altered immune function under the normal physiological conditions of the present experiment (67).

The lack of response may reflect the adequate nutritional and management conditions of the experiment, under which improved nutrient and phosphorus availability may be insufficient to alter immune function in the absence of nutritional or environmental stressors (67, 68).

The present findings agree with Fijabio et al. (32), who reported no significant effects of phytase on hematological or serum biochemical indices in broilers under normal rearing conditions.

Similarly, Al-Khalaifah et al. (69) found no significant effects of conventional vegetable oils on hematological or biochemical characteristics, while effects of dietary fatty acids on immunity may be more pronounced with long-chain n-3 PUFA than with conventional oils such as soybean or palm oil (70, 71).

Even though no statistically significant effects of treatment were found in this study, the interaction between the supplementation of phytase and the source of dietary oil regarding SRBC antibody titer ($P = 0.06$) and lymphocyte percentage ($P = 0.07$) came close to being significant. These tendencies might suggest subtle interactions between the availability of phosphorus and the composition of the dietary lipid which could become apparent when there is an immune challenge or in the case of larger experimental groups. Moreover, the absence of significant differences among dietary oil sources indicates that replacing soybean oil with palm oil did not compromise the measured immune responses of broiler chickens under the conditions of the present study.

Overall, phytase and dietary oil source had minimal effects on immune responses in healthy broilers under the conditions of the present study.

5. Conclusion

Phytase supplementation and dietary oil source interacted to influence growth performance and mineral digestibility, while neither phytase, oil source, nor their interaction adversely affected bone mineralization or immune responses. Partial or complete replacement of soybean oil with palm oil, particularly when combined with phytase, may therefore represent a practical feeding strategy for broilers without compromising feed efficiency, bone mineralization, or immunity. Given the lower cost of palm oil, its inclusion may also provide economic benefits; however, the optimal replacement level should be determined through cost–benefit analysis under commercial conditions.

Authors contribution:

Amir Ali Amiri contributed to the conception and design of the study, conducted the animal experiment, collected the samples, and contributed to data collection. Fatemeh Shirmohammad contributed to the study design, data analysis, interpretation of the results, and manuscript preparation. Morteza Mehri contributed to the experimental design, interpretation of the results, and critical revision of the manuscript. Farhad Moosakhani contributed to the laboratory analyses, interpretation of the results, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of interest:

The authors declare that they have no conflict of interest.

Data availability:

The data generated and/or analyzed during the current study are available from the corresponding author upon request.

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