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Effects of Herbal Vitamin D₃ and Phytase Supplementation to Broiler Feed on Performance, Bone Development and Serum Parameters of Broilers

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ABSTRACT

A trial was conducted to assess the effects of phytase supplementation and substitute Vitamin D₃ resource with Panbonis - a herbal vitamin D₃ source- (PAN) on performance, some carcass characteristics, tibia and serum parameters of broiler chickens. For this purpose, 11200 one-day-old, mixed sex (5600 male, 5600 female) Ross-308 chicks were administered 7 different diets based on corn, soybean and wheat throughout the 41-day trial. Dietary treatments; control group as T1 (5000 IU vitamin D₃), T2 (T1 + 500 FTU g⁻¹ phytase), T3 (3000 IU vitamin D₃ + 500 FTU g⁻¹ phytase + 100 mg kg⁻¹ PAN) and T4 (3000 IU vitamin D₃ + 500 FTU g⁻¹ phytase + 200 mg kg⁻¹PAN) were prepared to contain recommended levels of Ca-P however T5, T6 and T7 were formulated from T2, T3 and T4, respectively, by reducing 18% of Ca and P concentrations.

When overall results considered, there was no significant difference among treatments in terms of final live weight,

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mortality, weight gain, European Production Efficiency Factor (EPEF) and carcass parameters and mortality (P>0.05). While birds consuming diets containing phytase exhibited better FCR than control group without phytase (P<0.05), no additional improvement was obtained with PAN supplementation compared to other treatments without control group. Additionally partial replacement of PAN for synthetic form had no significant effect on tibia parameters and serum Ca, P levels even though serum Mg (in chicks fed sufficient Ca-P) and calcitriol were increased.

These results indicate that PAN could replace some part of synthetic vitamin D₃ without any adverse effect in broiler chickens. However, substitution rate of PAN in Ca and P deficient diets should be carefully studied more due to possible adverse effects on feed intake (12-41d) and weight gain (12-41d) as observed in the present study.

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1. Introduction

Vitamin D can derived from the diet or produced in the skin by means of sunlight. Dietary supplementation has become crucial due to intensive poultry farming not allowing substantial vitamin D synthesis. Vitamin D has been mostly provided to poultry by supplementation of the diet with synthetic forms of cholecalciferol (Vitamin D₃). Cholecalciferol must undergo change to form 25 hydroxycholecalciferol (25-OH-D₃) in liver and then in kidney 1,25- hydroxycholecalciferol (1,25-OH-D₃) which is active form of vitamin D₃. It has been long known that 1,25-OH-D₃ increases gut absorption of Ca-P therefore suboptimal vitamin D₃ levels in broiler diets adversely affect growth performance and bone development (Roberson & Edwards 1996; Biehl & Baker 1997). Besides, it was reported that enhanced phytate phosphorus availability through increasing gut mucosal phytase activity (Onyango et al 2006) thus decreased P excretion (Biehl & Baker 1997; Qian et al 1997) was obtained with supplementation

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of vitamin D₃ in broiler diets. Recently, there has been a growing interest in supplying vitamin D₃ with phytase in order to maximize utilization of phytate phosphorus. Some studies have evaluated combinations of phytase and the vitamin D analogs reported additive responses between those pair of additives. Snow et al (2004) obtained more bioavailable P release (0.07%) as a result of inclusion 1 α -OH vitamin D₃ (5 μ g) with phytase (300 IU) than addition of only 1 α -OH vitamin D₃ (0.04%) or phytase (0.02%) in broilers fed 0.75% Ca and 0.13% none phytate phosphorus (NPP) diets. This additive effect was also observed in weight gain and tibia ash (mg tibia⁻¹) in the same research (Snow et al 2004). Besides, Biehl et al (1995) demonstrated that utilization of Zn and Mn responded additively in combination of vitamin D₃ analogues with phytase.

Stressful conditions, such as high bird density, heat stress, mycotoxicosis, enteritis, malabsorption syndromes and certain immune disorders may impair liver or kidney hydroxylation of cholecalciferol so directly adding active form of vitamin D₃ could be assumed to be advantageous for broilers. Dietary supplementation with synthetic 1,25-vitamin D₃ is not practical commercially because of the high cost of chemical synthesis. Thus, herbal vitamin D₃ sources have been discussed as possible alternative to the synthetic counterparts. The plant *Solanum glaucophyllum* was established that contains the active form of vitamin D₃ in a glycosidic bound form by researchers (Wasserman et al 1976; Boland 1988). Studies have indicated that inclusion of *Solanum glaucophyllum* as vitamin D₃ source in diets either led some improvements or at least had no adverse effect on growth performance and bone development of broilers and eggshell thickness of laying hens (Morris et al 1977; Cheng et al 2004; Bachmann et al 2013). With these benefits in mind, it may suggested that inclusion of *Solanum glaucophyllum* with phytase in broiler diets gives an opportunity to reduce dietary Ca-P levels. However, there is a lack of information about the effect of herbal source of vitamin D₃ (PAN), reduced phosphorus and calcium level besides phytase presence.

Therefore this study was conducted to test the effects of PAN with the presence of phytase at 2 levels of dietary Ca and P on growth performance, carcass characteristics, tibia and some serum parameters of broiler chickens.

2. Material and Methods

2.1. Birds and housing

The research was carried out in broiler research house of Beypilic Broiler Company, one of the largest broiler integration, in Bolu province, Turkey. 11200 one-day-old, mixed sex (5600 male, 5600 female) Ross-308 chicks were weighed and randomly allocated to 56 floor pens (6.5 \times 2 m). Rearing conditions were set according to breeder guidelines (Anonymous 2014). Chicks were allowed *ad libitum* access to feed and water.

The research was conducted according to a completely randomized block design. Day old chicks were weighed and randomly distributed into 7 dietary treatments each has 8 replicates with 200 chicks in floor pens of experiment house through 41 days. Four phase feeding program was administered through the experiment as starter (0-11 days), grower (12-25 days), developer (26-35 days) and finisher (36-41 days).

2.2. Diets

All of the diets were formulated based on corn, soybean and wheat in pelleted form. Due to dietary additions of herbal vitamin D₃ were made at the expense of corn only compositions of diet 1, 2 and 5 were shown in Table 1. As could be seen in composition of diet 2 and 5, phytase addition to diets was made through considering Ca and P matrix values (Table 1). Diets for each feeding period were formulated to be isonitrogenous and isocaloric and to meet or exceed breeder guidelines (Anonymous 2014), but with different levels of available P (Pa), Ca and vitamin D₃ as given in Table 2. Starter feeds of all the treatments were produced as crumble form while grower and finisher feeds were pellet form in Beypilic Feed Mill.

Herbal Vitamin D₃ (Panbonis[®]-PAN): The herbal product contains 10 mg 1,25-Dihydroxycholecalciferol kg⁻¹ in glycoside form and recommended to use 50-500 g per ton feed in addition to vitamin D from other sources. The product was supplied from Herbonis Animal Health, Basel, Switzerland.

Phytase: Phyzyme[®] XP (5000 FTU phytase g⁻¹) from Danisco Animal Nutrition, Marlborough, UK. 600 g

phytase enzyme premix was included in 1 ton of diets to give 500 FTU g⁻¹ which assumed to release 0.11% Ca and 0.12% available P.

2.3. Data collection

All the procedures of animal use and biological material collection were approved by the Ethics Committee of Animal Use under the protocol number 2014-18-137. All chicks were weighed at first, 11th and 41st days of the research. Feed Intake was recorded for 0-11 and 12-41 days. Mortality was recorded on a daily basis. FCR was calculated for 0-11 and 12-41 days. EPEF (European Production Efficiency) was also calculated at the end of the experiment. At 24th days of age 2 chicks from each replicates near to average weight of the pen were selected, identified and killed for bone and blood serum measurements. Blood samples (1-2 mL) were collected by heart puncture and placed in test tubes, centrifuged for 10 minutes at 3000 rpm, and the serum was then placed in duly identified Eppendorf tubes and immediately frozen until analyses for serum alkaline phosphatase (ALP), creatine kinase (CK), calcitriol (CTL), Mg, P and Ca. The left tibia from each bird was excised, sealed in plastic bags and stored at -20 °C until further analysis. At the end of the trial 2 broilers from each pen was selected to assess carcass, drumsticks, breast meat yield. Carcass, drumsticks (with bones), and breast meat (with bones) were excised and weighed, then calculated as a percent of live body weight.

Table 1- Composition of basal diets; normal diet (T1), phytase applied diet with Ca and P matrixvalue (T2) and low Ca and P diet (T5) with phytase matrix applied, g kg⁻¹ air dry

Ingredients	Starter			Grower			Developer			Finisher		
	T1	T2	T5	T1	T2	T5	T1	T2	T5	T1	T2	T5
Corn	473.43	482.86	496.42	508.46	517.86	527.19	546.71	556.11	563.47	505.02	515.30	521.76
SBM ¹ , 46.5% CP	225.65	224.28	228.49	153.54	152.15	155.30	128.96	127.56	128.08	163.26	167.64	162.38
Full fat soybean	150.26	149.95	141.61	158.55	158.28	152.17	135.24	134.98	132.70	163.71	155.76	161.20
Wheat	50.00	50.00	50.00	70.00	70.00	70.00	70.00	70.00	70.00	85.00	85.00	85.00
Poultry meal	25.00	25.00	25.00	45.00	45.00	45.00	50.00	50.00	50.00	-	-	-
Soy oil	14.00	11.00	8.00	22.00	19.00	17.00	30.00	27.00	25.00	35.00	33.00	30.00
CGM ² , 60% CP	20.00	20.00	20.00	8.00	8.00	8.00	8.00	8.00	8.00	12.00	12.00	12.00
DCP	19.95	13.90	8.85	16.09	10.03	6.38	14.60	8.54	4.89	17.68	11.64	7.97
CaCO ₃	7.95	8.95	7.53	6.74	7.74	6.98	6.28	7.28	7.31	7.35	8.35	8.38
Choline Cl	0.37	0.36	0.36	0.28	0.28	0.28	0.25	0.25	0.25	0.36	0.36	0.35
Lysine HCl, 99%	3.37	3.24	3.29	2.61	2.49	2.53	1.99	1.87	1.90	1.50	1.38	1.40
DL-Methionine	3.32	3.16	3.15	2.53	2.37	2.37	1.97	1.81	1.80	2.42	2.27	2.26
Phytase	0.00	0.60	0.60	0.00	0.60	0.60	0.00	0.60	0.60	0.00	0.60	0.60
NaHCO ₃	1.80	1.80	1.80	1.50	1.50	1.50	1.40	1.40	1.40	2.20	2.20	2.20
NaCl	2.30	2.30	2.30	2.10	2.10	2.10	2.10	2.10	2.10	2.50	2.50	2.50
Salinomycin	0.40	0.40	0.40	0.40	0.40	0.40	0.50	0.50	0.50	-	-	-
Nicarbacin	0.20	0.20	0.20	0.20	0.20	0.20	-	-	-	-	-	-
Mineral premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated												
Crude protein, %	23.00	23.00	23.00	21.00	21.00	21.00	19.60	19.60	19.60	19.40	19.40	19.40
ME, kcal kg ⁻¹	3025	3025	3025	3150	3150	3150	3225	3225	3225	3225	3225	3225
Calcium, %	1.00	0.89	0.71	0.90	0.79	0.67	0.85	0.74	0.61	0.85	0.74	0.61
Total P, %	0.77	0.65	0.56	0.72	0.60	0.54	0.70	0.59	0.51	0.70	0.59	0.51
Available P, %	0.50	0.38	0.29	0.45	0.33	0.27	0.43	0.31	0.24	0.43	0.31	0.24
Met+Cys, %	1.07	1.07	1.07	0.95	0.95	0.95	0.86	0.86	0.86	0.86	0.86	0.86
Lysine, %	1.43	1.43	1.43	1.24	1.24	1.24	1.09	1.09	1.09	1.09	1.09	1.09
Analyzed												
Crude protein, %	23.15	23.05	22.95	20.96	20.98	21.15	19.40	19.45	19.62	19.55	19.45	19.32
ME, kcal kg ⁻¹	3032	3018	3034	3165	3146	3142	3240	3227	3231	3219	3217	3209
Calcium, %	1.04	0.90	0.70	0.93	0.81	0.68	0.84	0.73	0.63	0.85	0.75	0.62
Phosphorus, %	0.79	0.68	0.58	0.73	0.62	0.55	0.71	0.60	0.51	0.72	0.60	0.52

¹, soybean meal; ², corn gluten meal

Table 2- Experimental design

Treatments	Phytase FTUg ⁻¹	Herbal VitD ₃ , mg kg ⁻¹	VitD ₃ (IU)	Starter		Grower		Developer-Finisher	
				Ca, %	Pa ¹ %	Ca, %	Pa ¹ %	Ca, %	Pa ¹ %
T1	0	0	5000	1.00	0.50	0.90	0.45	0.85	0.43
T2	500	0	5000	0.89 (1.00)	0.38 (0.50)	0.79 (0.90)	0.33 (0.45)	0.74 (0.85)	0.31 (0.43)
T3	500	100	3000	0.89 (1.00)	0.38 (0.50)	0.79 (0.90)	0.33 (0.45)	0.74 (0.85)	0.31 (0.43)
T4	500	200	3000	0.89 (1.00)	0.38 (0.50)	0.79 (0.90)	0.33 (0.45)	0.74 (0.85)	0.31 (0.43)
T5	500	0	5000	0.71 (0.82)	0.29 (0.41)	0.67 (0.78)	0.27 (0.39)	0.61 (0.72)	0.24 (0.36)
T6	500	100	3000	0.71 (0.82)	0.29 (0.41)	0.67 (0.78)	0.27 (0.39)	0.61 (0.72)	0.24 (0.36)
T7	500	200	3000	0.71 (0.82)	0.29 (0.41)	0.67 (0.78)	0.27 (0.39)	0.61 (0.72)	0.24 (0.36)

Parenthesis shows the levels after phytase matrix values applied as 0.11% Ca and 0.12% available P; ¹, phosphorus available

2.4. Chemical analysis

Moisture, ash, crude protein (Nx6.25), ether extract, crude fiber analyses of feed ingredients and proximate analysis, sugar and starch of diets were performed according to the methods of the Association of Official Analytical Chemists (AOAC 2005). The ME content of the diets were calculated according to the equation (ME [kcal kg⁻¹]= 53+38×[% CP+2.25×% ether extractable fat {EE}+1.1×% Starch+% Sugar]) developed by Carpenter & Clegg (1956). Before analysis, meat and fat were gently removed from tibia bones. The bones were dried overnight at 100 °C, extracted in ether for 6 h, and burnt to ash in a muffle furnace at 600 °C. The ash from each tibia was used for phosphorus analysis according to AOAC (2005). The serum samples were analyzed by using of test-kits of Roche Diagnostics for serum calcium, phosphate, magnesium, alkaline phosphatase and creatine kinase. Serum 1.25-dihydroxyvitamin D₃ (CTL) were analyzed by using ELISA-kits of Immunodiagnostic, Bensheim Germany.

2.5. Statistical analysis

The data were analyzed as a completely randomized block design with 7 dietary treatments and 8 replicates using the ANOVA procedure of the MINITAB (Statistical Software, version 13). All percentage data were subjected to arcsine square root transformation. When significant differences among groups were found, means were separated using the Tukey HSD test.

3. Results and Discussion

3.1 Performance Parameters, feed cost, some carcass characteristics and tibia parameters

The effects of treatments on performance at days of 0-11 and 12-41 were presented in Table 3 and whole period in Table 4. As shown in Table 3, performance parameters of 0-11d were not affected by treatments (P>0.05). However at days of 11-41, phytase supplementation significantly improved FCR compared with control. Also replacement of PAN at any level in low Ca-P level (T5, T6) significantly (P<0.05) decreased feed intake and level of 100 mg kg⁻¹ replacement (T6) significantly decreased weight gain (P<0.05). As shown in Table 4 when whole growth period considered, there were no significant difference among treatments in terms of final live weight, weight gain, mortality and EPEF (P>0.05). On the other hand, phytase supplementation significantly improved FCR compared with control but, partial replacement of PAN for synthetic form had no improved effect on Feed Intake and FCR at any level.

The feed cost analysis for kg live weight given in table 4 showed that 100 mg kg⁻¹ PAN supplementation (T6) into diets containing low Ca and P in the presence of phytase has significantly reduced the feed cost for kg live weight (P<0.05) compared to other treatments, except T7. Although cost benefits mainly created by reduced level of Ca and P in T5, T6 and T7, growth performance and FCR obtained by 100 mg kg⁻¹ PAN supplementation had serious contribution in the feed cost reduction.

Table 3- Effects of herbal Vitamin D₃ and phytase supplementation on growth performance of broilers through 0-11 and 12-41 days

T*	Initial weights, g	Live weight 11 th day, g	Weight gain 0-11 days, g	FCR 0-11 days	Feed intake 0-11 days, g	Mortality 0-11 days	FCR 12-41 days	Feed intake 12-41 days, g	Weight gain 12-41 days, g
1	43.51±0.17	309.52±2.04	266.01±2.06	1.24±0.02	328.68±4.44	4.56±0.71	1.73±0.08a	4014.51±54.78a	2321.57±28.00ab
2	43.59±0.13	313.11±2.59	269.52±2.63	1.21±0.01	325.61±2.57	4.03±0.69	1.71±0.09b	4031.31±39.09a	2364.22±17.07a
3	43.57±0.11	312.20±1.75	268.63±1.73	1.23±0.01	330.28±2.90	4.00±0.77	1.70±0.08b	3992.86±32.93a	2351.14±21.20a
4	43.53±0.11	312.82±3.34	269.28±3.34	1.24±0.02	332.35±3.00	4.25±0.82	1.69±0.07b	4018.98±36.80a	2377.09±27.91a
5	43.51±0.18	310.49±2.94	266.98±2.89	1.22±0.02	324.38±4.54	3.56±0.65	1.69±0.09b	3963.47±29.36a	2343.41±16.43ab
6	43.61±0.16	315.59±2.56	271.98±2.61	1.22±0.01	332.05±2.96	6.38±0.85	1.70±0.08b	3835.96±33.84b	2261.21±19.81c
7	43.54±0.12	306.68±2.47	263.13±2.38	1.25±0.01	328.98±3.04	4.88±0.52	1.69±0.06b	3863.32±43.53b	2284.37±24.73bc
P	0.991	0.119	0.121	0.590	0.498	0.230	0.004	0.005	0.005

^{a-c}, means within a column with different superscripts are significantly different (P<0.05); *T, treatments

Table 4- Effects of herbal Vitamin D₃ and phytase supplementation on growth performance of broilers through 0-41 days and feed cost for kg live weight (FCFLW)

T*	Final Live Weight, 41 th day, g	Weight Gain 0-41 days	Feed Intake 0-41 days	FCR 0-41 days	Mortality 0-41 days, %	EPEF	FCFLW, TL
1	2731.11±24.57	2687.60±24.57	4506.79±43.80a	1.68±0.00a	10.81±0.78	340.31±3.59	2.69±0.03a
2	2729.20±15.77	2685.61±15.76	4436.91±28.14ab	1.65±0.00b	10.61±0.91	345.89±3.12	2.67±0.03ab
3	2729.13±9.54	2685.56±9.49	4428.08±21.97ab	1.65±0.00b	10.31±1.17	347.90±4.98	2.65±0.02ab
4	2742.09±25.19	2698.56±25.18	4432.82±31.70ab	1.64±0.00b	11.44±0.69	346.61±6.67	2.66±0.03ab
5	2711.68±29.59	2668.17±29.61	4378.11±41.88bc	1.64±0.00b	9.56±0.81	350.13±5.08	2.62±0.03ab
6	2727.67±23.38	2684.06±23.48	4413.60±37.81bc	1.64±0.00b	12.56±0.95	339.92±5.45	2.53±0.02c
7	2681.03±15.23	2637.49±15.28	4336.01±31.32c	1.64±0.00b	12.06±0.77	335.88±2.83	2.59±0.02bc
P	0.288	0.287	0,011	0.003	0.317	0.241	0.004

^{a-c}, means within a column with different superscripts are significantly different (P<0.05); *T, treatments

The effects of treatments on some carcass characteristics were presented in Table 5 and tibia parameters in Table 6. As shown Table 5 carcass characteristics were not affected by treatments. As shown Table 6, phytase supplementation significantly decreased tibia P (% of dry-defatted tibia) and tibia ash (% of DM) compared with control (P<0.05) but partial replacement of PAN for synthetic form had no effect on tibia parameters.

Performance data showed that synthetic vitamin D₃ levels might be reduced by PAN addition without any adverse effect on weight gain, feed intake and FCR during the starter phase. However; from d 12-41, replacing 2000 IU synthetic vitamin D₃ with PAN in reduced Ca-P diets led to significantly decreased feed intake (P<0.05). In the same growth phase, it was also observed that chicks fed insufficient Ca-P needed 200 mg kg⁻¹ PAN to maintain weight gain as 100 mg kg⁻¹ PAN was adequate in broilers fed sufficiently in Ca-P. This result verifying other studies indicating that chicks needed more vitamin D₃ when suboptimal concentrations of Ca-P contents in diets (Baker et al 1998; Whitehead et al 2004; Rama Rao et al 2006). The present study in line with Cheng et al (2004) that reported no additional benefit in terms of FCR and weight gain by inclusion of *Solanum glaucophyllum* (dried leaves 7.5 g kg⁻¹) combination with phytase (1200 FTU kg⁻¹) in 0.59% Ca and 0.19% NPP containing diets. Addition to live weight, Bachmann et al (2013) found no significant (P>0.05) difference between product based on dried *Solanum glaucophyllum* leaves (providing 10 µg of 1,25- OH vitamin D₃) and free 1,25- OH vitamin D₃ (2.5 and 5 µg) in terms of tibia strength and stiffness in broiler chickens. Similarly, in the present study, tibia and carcass parameters of chicks fed diets that contain lower vitamin D₃ level (3000 IU kg⁻¹ feed) with PAN were similar to that of the chicks fed diets contain regular vitamin D₃ level (5000 IU kg⁻¹ feed) without PAN (P> 0.05). These results indicated that PAN could replace synthetic one in broiler diets without any adverse effect on tibia and carcass parameters. Phytase addition to diets including regular Ca-P levels did not significantly affect weight gain, FCR and mortality during the starter period (P>0.05). Chicks given the low Ca-P diets with phytase had

similar weight gain and FCR when compared to birds consume regular Ca-P diets in the same period. This is in line with previous studies that indicate Ca-P level could be reduced by adding phytase without any depression in performance (Broz et al 1994; Sebastian et al 1996). Phytase addition improved FCR but feed intake and weight gain were depressed by reducing Ca-P level regardless of phytase or PAN supplementation during d 12-41 ($P < 0.05$). Although tibia ash (% of DM) and tibia P (% of dry-defatted tibia) were negatively affected by decreasing levels of Ca and P in the diets, performance of broilers was not affected in general throughout study. Therefore, it could be claimed that Ca and P requirements of broilers needed to be carefully further studied, and re-evaluated.

Table 5- Effects of herbal Vitamin D₃ and phytase supplementation on some carcass characteristics % of live body weight

Treatments	Carcass yield	Drumsticks	Breast meat
1	71.78±0.44	31.59±0.27	32.58±0.38
2	71.89±0.45	32.03±0.29	32.08±0.37
3	72.13±0.35	32.00±0.20	32.31±0.36
4	71.87±0.34	31.36±0.25	32.81±0.36
5	72.67±0.64	32.18±0.31	32.46±0.45
6	72.28±0.35	31.57±0.30	32.86±0.34
7	71.63±0.54	31.65±0.31	32.21±0.44
P	0.733	0.321	0.727

Table 6- Effects of herbal Vitamin D₃ and phytase supplementation on tibia parameters

Treatments	Dry-defatted tibia weight, g	Tibia ash, % of DM	Tibia P, % of ash	Tibia P, % of dry-defatted tibia	Tibia ratio, % in 24 day's live weight
1	3.97±0.16	41.83±0.44a	17.13±0.17	7.18±0.12a	0.31±0.01
2	4.00±0.11	40.59±0.30b	16.94±0.14	6.89±0.09b	0.30±0.01
3	3.74±0.11	40.60±0.28b	16.97±0.09	6.90±0.06b	0.29±0.00
4	3.93±0.13	40.60±0.28b	17.03±0.07	6.92±0.07b	0.31±0.01
5	3.83±0.11	39.95±0.38bc	17.01±0.09	6.81±0.05b	0.30±0.00
6	3.60±0.11	39.77±0.25bc	17.05±0.08	6.80±0.06b	0.29±0.01
7	3.73±0.11	39.64±0.31c	16.98±0.06	6.74±0.05b	0.30±0.01
P	0.217	0.002	0.920	0.011	0.442

^{a-c}, means within a column with different superscripts are significantly different ($P < 0.05$)

3.2. Serum parameters

The effects of treatments on some serum parameters were presented in Table 7. As shown in Table 7, while serum calcium, ALP and CK (except T2) concentrations were not significantly affected by treatments, serum CTL and Mg concentrations were increased with PAN replacement in broilers fed Ca-P adequately and P concentration was increased with phytase supplementation in broilers fed Ca-P adequately.

Table 7- Effects of herbal Vitamin D₃ and phytase supplementation on serum parameters

Treatments	Calcium (mmol L ⁻¹)	Phosphate (mmol L ⁻¹)	Magnesium (mmol L ⁻¹)	ALP ¹ (U l ⁻¹)	CK ² (U l ⁻¹)	1,25-Dihydroxy Vit D ₃ (pg mL ⁻¹)
1	1.72±0.06	2.13±0.04d	0.73±0.04c	10.78±1.59	17.55±1.68b	118.81±6.82bc
2	1.79±0.08	2.34±0.04ab	0.74±0.05c	11.73±1.73	22.43±1.27a	113.50±4.83cd
3	1.74±0.08	2.40±0.06ab	0.95±0.05b	13.48±1.46	16.32±1.38b	125.89±6.53bc
4	1.70±0.06	2.45±0.06a	1.13±0.08a	8.05±1.07	17.24±1.74b	133.91±4.91ab
5	1.53±0.05	2.20±0.06cd	1.07±0.05ab	10.74±1.55	17.13±1.77b	142.45±6.56a
6	1.66±0.09	2.27±0.07bc	1.02±0.07ab	12.72±1.30	15.29±1.53b	98.74±4.86d
7	1.65±0.09	2.22±0.08cd	1.16±0.08a	9.94±1.56	17.87±1.80b	101.91±5.49d
P	0.243	0.001	0.000	0.114	0.051	0.000

¹, alkaline phosphatase; ², creatine kinase; ^{a-d}, means within a column with different superscripts are significantly different ($P < 0.05$)

It has previously been demonstrated that the plant *Solanum glaucophyllum* contain vitamin D₃ as mainly derivatives of 1,25-OH-vitamin D₃ glycosidically-linked to carbohydrate units (Skliar et al 1992). Earlier studies evaluating the effects of *Solanum glaucophyllum* have found markedly increase in serum Ca (Uribe et al 1974) and 1,25-OH vitamin D₃ (Napoli et al 1977) concentration in rats, elevations of serum Ca and P levels in rabbits (Mautalen 1972). Similarly, in the present study, despite reduced synthetic vitamin D₃ levels in diet chicks were able maintain serum Ca and even tend to increase phosphate concentration by providing PAN. Moreover, enhancement of serum CTL and magnesium levels were detected in broilers fed sufficient Ca-P with PAN addition (P<0.05). This increase in serum Mg level could be thought as a result of increased intestinal phytase activity by providing PAN which was previously reported that synthetic vitamin D₃ derivatives as responsible (Biehl et al 1995; Onyango et al 2006). However, no significant increase observed in serum Ca and P levels in chicks fed PAN which may be hypothesized that Ca-P demand for bone tissue prevented significant increase of serum Ca-P concentrations in broiler chickens. In accordance with previous studies (Gray & Garthwaite 1985; Cheng et al 2004) reported that feeding with Ca-P deficient diets could stimulate renal production of CTL was observed when compared T2 and T5 in the present study. However, substitution of a particular proportion of PAN at the expense of synthetic source caused that decreased serum CTL levels (P<0.01) as serum Mg concentrations remained similar in birds fed lower Ca-P diets. The mechanism behind the decreased CTL levels with PAN is not clear but could be related lowered renal CTL synthesis by providing PAN. Moreover, it may be postulated that increasing metabolic consumption of CTL to maintain.

4. Conclusions

Considering the growth performance, feed cost for kg live weight, carcass, tibia and serum parameters it might be concluded that 100 mg kg⁻¹ herbal source of vitamin D₃ (PAN) could be substituted for a portion of synthetic vitamin D₃ without any adverse effect and would have an economic contribution because of significant feed cost benefit in the presence of phytase at low Ca and P broiler diets. However, 200 mg kg⁻¹ supplementation of PAN at low Ca and P diets must be carefully considered and further studied because of negative impact on feed intake and tibia ash found in the present study. The results of the present study were also showed that phytase supplementation of broiler diets could allow to reduce P and Ca level without any adverse effects on growth performance, carcass and serum parameters by giving economic benefits for feed cost.

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