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Supplementation of Microbial and Fungal Phytases to Low Protein and Energy Diets: Effects on Productive Performance, Nutrient Digestibility, and Blood Profiles of Broilers

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Abstract: To evaluate in possible use of phytases for improving the utilization of low protein and energy diets, 420, one-day-old chicks were distributed among 7 groups (5 replicates of 12 chicks/group). During the starter (1–35 day), grower (37–56 day), and finisher (57–64 day) periods, the control group fed diets containing 21.2% crude protein (CP)-2947 Kcal/kg metabolizable energy (ME), 19.6 CP-3023 ME and 18.0 CP-3100 ME, respectively. The three low-CP groups received diets isocaloric but with −1% CP than the control, while the three low-CPME groups fed diets with −1% CP and −100 Kcal than the control. In addition, the low-CP and low-CPME groups were supplemented with 0 (low-CP_uns and low-CPME_uns), 500 U/kg of an *Aspergillus niger* (low-CP_AP and low-CPME_AP) or 500 FTU/kg of an *Escherichia coli* phytase (low-CP_EP and low-CPME_EP), respectively. Low-CP and low-CPME diets decreased ($p < 0.01$) the intake of feed as well as the protein and metabolizable energy conversion ratios in comparison to the control group. In general, phytases lowered ($p < 0.01$) the intake of feed, protein, and energy, but bacterial phytase showed a higher ($p < 0.01$) effect than *A. niger* one. The diets with low-CP and low-CPME levels decreased ($p < 0.01$) the amount of the excreta nitrogen. The supplementation of phytases had similar effects on digestibility of nutrients, carcass traits, bone mineralization and blood biochemistry. The supplementation of *A. niger* increased abdominal fat deposition of compared low-CPME diet compared to low-CPME_uns diet. All diets showed similar production index allowing the use of low-CPME diet when phytases was supplemented.

Keywords: phytase; broiler; low energy and protein diets; productive performance; blood profiles

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1. Introduction

One of the challenges in poultry production is to reduce the level of proteins and energy in the diets, to improve the sustainability and reduce the costs of the production. A possible approach could be the use of diets containing lower crude protein (CP) and metabolizable energy (ME) compared to the standard corn-soybean meal-based diets. However, the use of low-CP and ME diets can decrease body weight gain, impair feed conversion ratio, and increase fat deposition [1]. The low-CP diets can be improved by essential amino acids supplementation, avoiding the impairment of the growth performance also under poor sanitary conditions [2]. However, Liu et al. [3] indicated that balancing amino acids requirements of poultry is problematic, and an alternative promising strategy could be condensing starch: protein ratios. This goal could be reached by using enzyme supplementations.

The use of phytase is an exciting approach as this enzyme had a positive effect on mineral and non-mineral nutrient availability. The effects of phytase are linked to its activity on phytic acid: removing this anti-nutritional factor improves the nutritional value of the diet in terms of mineral bioavailability and energy and protein efficiency [4]. In the last years, there were several evidences of the effect of phytase on improving nutrient availability. Lu et al. [5] observed that phytase increased the expression of GLUT2 in pigs, and this can indicate an upregulation of glucose absorption from the intestine by phytase. Ren et al. [6] observed that increasing phytase (*E. coli*) levels in the diets of pigs increased ($p < 0.05$) the apparent ileal digestibility of some amino acids (Arg, Lys, His, Asp, Ile, Trp, and Glu). Babatunde et al. [7] showed that phytases from *Aspergillus niger*, *A. oryzae* and *Trichoderma reesei* can improve growth performance, nutrient, and mineral digestibility of broiler. Ennis et al. [8] evaluated the inclusion of phytase and carbohydrase as a possible strategy to optimize low-energy diets in male broiler and observed that supplementing phytase at a 1500 FTU/kg enhances broiler performance by improving 28 to 44 and 0 to 44 days FCR by 4 and 3 points, respectively.

The phytases commonly used in poultry nutrition can have different origin, and thus mode of action. Under a chemical point of view, the phytase is an enzyme, a phosphatase, able to hydrolyze phosphate groups. According to the position of the phosphate group in the myo-inositol ring, they can be classified as 3-phytase and 6-phytase [9]. Phytases can differ in terms of optimal pH, resistance to digestive enzymes, and thermostability [10]. Standing these considerations, it is clear that there are different commercial products containing different kinds of phytase. This paper aimed to compare the effect of 3 and 6-phytase supplementation on productive performance, meat quality, nutrient utilization, and production cost of colored slow-growing broilers fed corn-soybean diets with low CP and ME concentrations.

2. Materials and Methods

All the animals were treated according to the principles of the animal welfare stated by the Directive 63/2010/EEC regarding the protection of the animals used for experimental and other scientific purposes. The experimental procedures were approved by the Ethical Animal Care and Use Committee of the Department of Veterinary Medicine and Animal Production of the University of Napoli Federico II, Italy (prot. N. 2017/0017676).

2.1. Experimental Chickens Design and Diets

During 1 to 64 day of age, 420, one-day-old colored slow-growing broiler chicks from Sasso strain (sex ratio 1:1) were divided into 7 groups (5 replicates of 12 chicks each). Each replicate was housed in a floor pen (1.0 m × 1.0 m/pen) with a rice hulls litter. The groups were fed different starter (1–35 d), grower (37–56 d) and finisher (57–64 d) mash diets according to the broiler's age. Along the experimental period, the control group was fed three diets with the following contents of crude protein (CP) and metabolizable energy (ME): 21.2% and 2947 kcal/kg; 19.6% and 3023 kcal/kg; 18.0% and 3100 kcal/kg, respectively, in the starter, grower, and finisher period. The 3 low-CP groups were fed diets with similar ME contents than the control but in which CP percentages were reduced by around 1%. The 3 low-CP groups were submitted to 3 different dietary treatments as follows: low-CP unsupplemented group (low-CP_uns); low-CP fungal phytase group, supplemented with 500 U/kg of a diet of a fungal phytase (low-cp_FP group, *Aspergillus niger* phytase, Natuphos® BASF, Germany) and low-CP bacterial phytase group, supplemented with 500 FTU/kg diet of a bacterial phytase (low-CB_BP group, *Escherichia coli* phytase, Phyzyme® Danisco Animal Nutrition). The other 3 groups were fed low-CP and ME diets (low-CPME) in which the percentage of protein and the amount of energy were reduced by 1% and 100 kcal, respectively, in comparison to the control diets. The 3 low-CPME groups were submitted to the same treatments as the low-CP groups: low-CPME_uns, low-CPME_FP and low-CPME_BF groups.

The diets (Table 1) were formulated according to NRC [11]. The percentages of Ca and available P of the diets supplemented with phytase were not corrected for the phytase equivalent value to test the effect of the two phytases on the utilization of protein and energy when sufficient levels of Ca and P are available in the diets. Chemical analyses of diets agreed to Association of Official Analytical Chemists (AOAC) [12]. Diets and water were administered ad libitum along the trial. Chicks were submitted to a light:dark cycle of 23:1 along the entire period of the trial. Vaccinations and medical care were in line with the common veterinary practice for broilers. All the chicks have been raised under the same managerial, hygienic, and environmental conditions.

Table 1. Ingredients and chemical-nutritional characteristics of the diets used along the trial.

	Starter Diets			**Grower Diets**			**Finisher Diets**		
	Control	**Low-CP**	**Low-CPME**	**Control**	**Low-CP**	**Low-CPME**	**Control**	**Low-CP**	**Low-CPME**
					Ingredients g/kg				
Yellow corn	583.5	585.0	585.0	626.5	626.5	626.5	630.0	630.0	630.0
Soybean meal	320.0	300.0	300.0	275.0	255.0	255.0	282.5	260.0	260.0
Fish meal	30.0	30.0	30.0	30.0	30.0	30.0	-	-	-
Limestone	10.0	10.0	10.0	9.00	9.00	9.00	9.00	9.00	9.00
Dicalcium phosphate	18.0	18.0	18.0	16.0	16.5	16.5	17.00	17.0	17.0
Vit + Min Premix [1]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
NaCl	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
DL-Methionine	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
L-Lysine (HCl)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vegetable oils	30.0	37.0	22.0	35.0	42.0	26.5	53.0	61.0	46.00
Washed building sand	-	11.5	26.5	-	12.5	28.0	-	14.5	29.50
					Chemical-Nutritional Characteristics				
Dry matter [2]	89.61	89.70	89.53	89.87	89.63	89.76	89.57	89.69	89.84
ME, MJ/Kg [3]	12.34	12.36	11.95	12.66	12.66	12.24	12.94	12.95	12.54
CP % [2]	21.03	20.01	20.02	19.49	18.51	18.84	17.80	16.79	16.81
Methionine % [3]	0.51	0.50	0.50	0.49	0.48	0.48	0.44	0.42	0.42
SAA % [3]	0.85	0.82	0.82	0.80	0.78	0.78	0.74	0.71	0.70
Lysine % [3]	1.24	1.19	1.19	1.13	1.08	1.08	1.00	0.94	0.94
Calcium % [3]	0.99	0.99	0.99	0.90	0.90	0.90	0.81	0.80	0.80
Av. P % [3]	0.49	0.49	0.49	0.45	0.45	0.45	0.40	0.39	0.39
Crude fat % [2]	5.47	5.98	4.70	6.21	6.74	5.26	7.63	7.98	6.97
Crude fiber % [2]	3.47	3.34	3.39	3.31	3.24	3.20	3.33	3.24	3.26
Ash % [2]	9.24	10.02	11.21	9.30	10.28	10.19	9.18	10.19	11.63
NFE % [2]	60.79	60.65	60.68	61.69	61.23	62.87	62.06	61.80	61.33

[1] Vit + Min mixture provides per kg: vitamin A (retinyl acetate) 24 mg, vitamin E (dL-tocopheryl acetate) 20 mg, menadione 2.3 mg, Vitamin D3 (cholecalciferol) 0.05 mg, riboflavin 5.5 mg, calcium pantothenate 12 mg, nicotinic acid 50 mg, choline chloride 600 mg, vitamin B12 10 g, vitamin B6 3 mg, thiamine 3 mg, folic acid 1 mg, d biotin 0.50 mg. Trace mineral (milligrams per kilogram of diet): Mn 80 Zn 60, Fe 35, Cu 8, Se 0.60. [2] Analyzed values. [3] Calculated values. SAA—sulphur-containing amino-acids; NFE—nitrogen-free extracts.

Chicks were individually weighed at the beginning (1 d) and at the end of the trial (64 d), and body weight gain (BWG) was calculated for the interval 1–64 days of age. At the same days, feed intake has been measured and used to calculate the feed conversion ratio (FCR). The conversion ratios of protein (PCR) and energy (ECR) intakes were calculated as protein (g) or ME (Kcal) necessary to gain one gram of BWG. The survival rate of chicks was monitored daily along the trial.

2.2. Digestibility Trial

The coefficients of the apparent nutrient digestibility of the diets were measured at 64 d of age using 6 male birds per treatment (2 broiler from 3 replicates) to avoid the confound

effect of sex on the digestibility of nutrients, according to Attia et al. [13]. Fecal and urinary nitrogen in excreta samples have been separated according to Jakobsen et al. [14]. The chemical analysis of diets and excreta were determined according to AOAC [12]. The apparent digestibility of the nutrients (dry matter, crude protein, crude fiber, and crude fat) as well as the apparent ash retention were calculated as the ratio between the daily retained nutrient (g/day) and its daily intake (g).

2.3. Slaughter Test

At 64 days of age, 6 broilers (3 males and 3 females) representative of each replicate were weighed after overnight fasting, and then slaughtered. After plucking and removing the inedible parts (head, legs, and viscera), the whole carcasses (dressed carcasses), and their front and hind parts were weighed. The inner organs (liver, pancreas, and spleen) were weighed, and the intestinal and caecal length were measured and expressed as percentage of the live weight. The total visceral fat, including those located in the abdominal cavity (AF) and surrounding the intestines and heart, were separated, weighed, and expressed as a percentage of live weight.

The right tibia of the 6 slaughtered broilers was separated, cleaned in hexane for 48 h to remove fat, and thus dried in a heater until constant weight was reached. The length (mm), width (mm), and weight (g) were measured. The percentage of ash in the defatted tibia and the contents of Ca and P were measured according to AOAC [12].

At 64 days of age, samples of blood were collected from the wing vein of 6 broilers per treatment (representative of each replicate) in heparinzed tubes. After the separation of plasma by centrifugation (3000 rpm for 15 min) the concentrations of Ca, inorganic P and alkaline phosphatase were measured as described by Attia et al. [15]. The biochemical constituents of blood plasma (total protein, albumin, aspartate aminotransferase (AST), total lipids, cholesterol, and alkaline phosphatase) were measured using specific diagnosing kits (Diamond Diagnostics Company, Egypt) according to Attia et al. [16]. The amount of the globulin was calculated as total protein-albumin.

The European Production Index (EPI) has been calculated according to the following formula: livability % × BW (kg)/age (d) × FCR × 100.

2.4. Statistical Analysis

Before running the statistical analysis, the normality of the error distribution as well as data were tested with Shapiro–Wilks test for normality [17]. The four assumption of ANOVA were validated according to random selection of the samples. In addition, the homogeneity of the variance (homoscedasticity) has been evaluated using the Levene's test [17]. Data were analyzed by the GLM procedure of Statistical Analysis Software [17] using two-way factorial design (3 types of diets by 2 types of phytase besides the positive control). The following model was used: Yijk = μ + SDi + DTj + (D × DT)ij+ eijk, where Yijk = the dependent variables; μ = general mean; SDi = effect of types of diet; DTj = effect of different phytases; (D × DT)ij = effect of the interaction between types of dies and phytases; and eijk = random error. The pen (replicate) was the experimental unit for the growing performance, while the single bird was the experimental unit for nutrient digestibility, carcass and meat traits, and blood profiles. Mean differences at $p < 0.05$ has been tested using the Student–Newman–Keuls test. Survival rate has been analyzed by chi-square test.

3. Results

Table 2 reports the growth performance of slow-growing broilers from 1 to 64 days of age. Both low-CP and low-CPME diets did not affect BWG, final weight, and FCR of broilers; however, these diets similarly reduced ($p < 0.01$) feed and protein intake, and protein and metabolizable energy conversion ratios in comparison to the control group. The energy intake was the lowest ($p < 0.01$) in broilers fed low-CPME diets, followed by low-CP and control diets. The survival rate was not different among groups. The use of

phytase decreased ($p < 0.01$) feed, protein, and energy intake; in this regard, the *E. coli* phytase had a greater ($p < 0.01$) effect than *A. niger* one.

Table 2. Growth performance of broilers as affected by diet, type of phytase, and their interaction.

	BWG g	Final BW g	Feed Intake g	Protein Intake g	Energy Intake Kcal	FCR g/g	PCR g/g	ECR Kcal/g	Mortality n
Effect of Diet									
Control	1757	1802	4024 [a]	788 [a]	12130 [a]	2.29	0.621 [a]	7.85 [a]	0
Low-CP	1661	1694	3824 [b]	711 [b]	11531 [b]	2.32	0.518 [b]	7.15 [b]	2
Low-CPME	1649	1706	3813 [b]	709 [b]	11125 [c]	2.31	0.511 [b]	6.86 [b]	2
Effect of Type of Phytase									
FP	1653	1698	3825 [a]	710 [a]	11,335 [a]	2.33	0.517	7.06	1
BP	1623	1667	3727 [b]	693 [b]	11,056 [b]	2.30	0.521	6.99	3
Interaction Diet × Phytase									
Control	1758	1802	4024 [a]	788 [a]	12,130 [a]	2.29	0.621	7.85	0
Low-CP × Uns	1702	1748	3959 [b]	736 [b]	11,194 [c]	2.33	0.510	7.11	0
Low-CP × FP	1632	1675	3804 [c, d]	708 [c, d]	11,472 [b]	2.33	0.512	7.20	1
Low-CP × BP	1614	1659	3707 [d]	690 [d]	11,178 [c]	2.30	0.532	7.14	1
Low-CPME × Uns	1674	1720	3853 [c]	716 [c]	11,244 [c]	2.30	0.503	6.81	0
Low-CPME × FP	1677	1722	3837 [c]	713 [c]	11,197 [c]	2.32	0.521	6.93	2
Low-CPME × BP	1632	1676	3748 [d]	697 [d]	10,933 [d]	2.31	0.509	6.85	0
SEM	82.8	84.5	18.5	3.41	55.0	0.037	0.012	0.146	—
***p* Values**									
Diet	NS	NS	0.0001	0.0001	0.0001	NS	0.001	0.008	—
Phytase type	NS	NS	0.0001	0.0001	0.0001	NS	NS	NS	—
Interaction	NS	NS	0.005	0.005	0.004	NS	NS	NS	—

[a], [b], [c], [d] means within a column with different superscripts are significantly different ($p < 0.05$); FP—fungal phytase; BP—bacterial phytase; Uns—unsupplemented; SEM—standard error or mean; NS—not significant. Low-CP—low crude protein diet; low-CPME—low crude protein and metabolizable energy diet; FCR—feed conversion ratio; PCR—protein conversion ratio; ECR—energy conversion ratio.

An effect of the interaction diet × type of phytase was detected ($p < 0.01$) for feed, energy, and protein intake. The control group showed the highest values for the three criteria; however, in the low-CP groups, the use of both phytases similarly decreased ($p < 0.01$) the feed and protein intake, while in the low-CPME diets, bacterial phytase decreased both feed and protein intake in comparison to the other two groups. Regarding the energy intake, in the low-CP groups, the fungal phytase gave the highest values while in the low-CPME diets, fungal phytase gave results not different from the unsupplemented group and higher than the bacterial phytase group.

Mortality was determined as number of dead birds ranged from 0 to 2 birds per experimental group (Table 2), with the highest incidence in the group fed a low-CPME diet supplemented with fungal phytase followed by those fed a low-CP diet supplemented with fungal and bacterial phytase.

The results of digestibility trial (Table 3) showed that low-CPME diets decreased ($p < 0.01$) nitrogen excreta in comparison to the control and low-CP groups. The types of phytases did not affect the dry matter, crude fiber, and crude protein digestibilities as well as the percentage of ash retention and the percentage of nitrogen in the feces. There were no interactions of diet × type of phytase for the criteria summarized in Table 3.

Table 3. Effect of diet, type of phytase, and their interaction on nutrient digestibility, ash retention and fate of nitrogen.

	Digestibility %					Nitrogen %	
	DM	**CP**	**CF**	**EE**	**Ash retention %**	**Excreta**	**Feces**
Effect of Diet							
Control	80.4	75.5	29.0	79.6	31.4	5.41 [a]	2.52
Low-CP	81.3	77.5	31.4	79.9	32.6	5.05 [a]	2.37
Low-CPME	80.5	77.1	31.2	80.5	32.6	5.09 [b]	2.39
Effect of Type of Phytase							
FP	82.3	78.0	33.1	80.7	33.0	5.08	2.26
BP	82.0	78.2	32.3	80.7	33.3	5.04	2.27
Interaction Diet × Phytase							
Control	80.4	75.5	29.0	79.6	31.4	5.41	2.52
Low-CP × Uns	79.1	76.0	29.0	78.8	31.4	5.14	2.55
Low-CP × FP	82.4	78.1	33.1	80.4	33.0	5.02	2.26
Low-CP × BP	82.4	78.6	32.1	80.5	33.4	5.01	2.28
Low-CPME × Uns	77.9	75.5	28.0	79.7	31.5	5.06	2.61
Low-CPME × FP	82.1	78.0	33.1	81.0	33.0	5.14	2.26
Low-CPME × BP	81.5	77.9	32.5	81.0	33.2	5.07	2.27
SEM	0.59	0.45	0.90	0.91	0.44	0.09	0.04
***p* Values**							
Diet	NS	NS	NS	NS	NS	0.01	NS
Phytase type	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS

[a], [b] means within a column with different superscripts are significantly different ($p < 0.05$); FP—fungal phytase; BP— bacterial phytase; Uns—unsupplemented; SEM—standard error or mean; NS—not significant; low-CP— low crude protein diet; low-CPME—low crude protein and metabolizable energy diet.

The effects of diet and type of phytase on carcass traits are presented in Table 4. Low-CP diets negatively affected ($p < 0.01$) the percentage of intestinal length compared to the control group but low-CPME diets showed no differences in comparison to the other two groups. The use of both phytases did not affect carcass traits. There was, for the percentage of abdominal fat, an effect of the interaction ($p < 0.05$): broilers fed low-CPME diets had a higher percentage of abdominal fat when fungal phytase was added in comparison to the control group.

Tibia characteristics and concentrations of Ca and P in the blood plasma of broilers are presented in Table 5. No effects were observed due to diet CP and ME levels, and types of phytase. No effect of the interaction between the two main effects was observed for the data reported in Table 5.

The effect of diet and type of phytase on total protein, albumin, globulin, total lipids, cholesterol, alkaline phosphatase, and aspartate aminotransferase of the blood of slow-growing broilers are reported in Table 6. Low-CP and low-CPME diets similarly increased ($p < 0.01$) the albumin than the control group. The use of both types of phytase did not affect the tested blood biochemistry. No effects of the interaction diet × type of phytase were observed.

Table 4. Effect of diet, type of phytase, and their interaction on carcass traits and production index (%) of broilers.

	Dressing	AF	Liver	Pancreas	Spleen	Intestinal Lenght	Caecal Lenght	EPI
Effect of Diet								
Control	70.9	1.93	2.40	0.220	0.165	9.48 ^{a}	0.876	169
Low-CP	68.4	2.18	2.10	0.200	0.171	8.18 ^{b}	0.938	158
Low-CPME	69.7	2.28	2.17	0.230	0.162	8.73 ^{a,b}	0.939	157
Effect of Type of Phytase								
FP	67.9	2.47	2.07	0.220	0.165	8.65	0.963	155
BP	69.6	2.66	2.17	0.200	0.165	8.99	0.924	156
Interaction Diet × Phytase								
Control	70.9	1.93 ^{b}	2.40	0.220	0.165	9.48	0.875	169
Low-CP × Uns	68.6	1.68 ^{b}	1.92	0.212	0.169	7.55	0.989	162
Low-CP × FP	67.1	2.02 ^{b}	2.29	0.206	0.147	7.96	0.938	154
Low-CP × BP	69.4	2.84 ^{a,b}	2.08	0.179	0.197	9.02	0.885	155
Low-CPME × Uns	70.6	1.44 ^{b}	2.05	0.249	0.154	7.91	0.868	161
Low-CPME × FP	68.6	3.30 ^{a}	2.06	0.245	0.182	9.32	0.987	157
Low-CPME × BP	69.9	2.10 ^{a,b}	2.05	0.194	0.150	8.92	0.962	157
SEM	1.20	0.379	0.147	0.020	0.018	0.489	0.056	5.71
***p* Values**								
Diet	NS	NS	NS	NS	NS	0.01	NS	NS
Phytase type	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	0.05	NS	NS	NS	NS	NS	NS

^{a}, ^{b} means within a column with different superscripts are significantly different ($p < 0.05$); FP—fungal phytase; BP— bacterial phytase; Uns—unsupplemented; SEM—standard error or mean; NS—not significant; low-CP—low crude protein diet; low-CPME—low crude protein and metabolizable energy diet; AF: abdominal fat; EPI— European production index.

Table 5. Effect of diet, type of phytase and their interaction on tibia characteristics, and plasma calcium and inorganic phosphorus of broilers.

	Tibia Characterstics					Plasma	
	Lenght mm	Weight g	Diameter mm	Ash %	Calcium %	Phosphorus %	Calcium mg/dL
Effect of Diet							
Control	110	7.59	11.7	44.7	20.3	10.2	10.6
Low-CP	110	8.01	11.4	44.7	20.6	10.2	11.2
Low-CPME	109	8.06	11.5	44.9	20.8	10.4	11.3
Effect of Type of Phytase							
FP	110	8.32	11.5	44.9	20.8	10.3	11.6
BP	111	7.98	11.8	45.2	20.9	10.4	11.9
Interaction Diet × Phytase							
Control	110	7.59	11.7	44.8	20.3	10.2	10.6
Low-CP × Uns	108	7.52	10.6	44.5	20.4	10.1	10.3
Low-CP × FP	112	8.65	11.9	44.7	20.5	10.1	11.5
Low-CP × BP	111	7.87	11.7	45.0	20.8	10.3	11.8
Low-CPME × Uns	109	7.92	11.4	44.1	20.2	10.1	10.0
Low-CPME × FP	108	7.99	11.2	45.2	21.2	10.5	11.8
Low-CPME × BP	111	8.10	11.2	45.4	20.9	10.5	12.1
SEM	2.20	0.66	0.50	0.41	0.31	0.16	0.346
***p* Values**							
Diet	NS	NS	NS	NS	NS	NS	NS
Phytase type	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS

FP—fungal phytase; BP, bacterial phytase; Uns—unsupplemented; SEM—standard error or mean; NS—not significant; low-CP—low-crude protein diet; low-CPME—low crude protein and metabolizable energy diet.

Table 6. Effect of diet, type of phytase, and their interaction on blood traits of broilers.

	TP g/dL	Alb g/dL	Glob g/dL	TL mg/dL	Chol mg/dL	AP U/L	AST U/L
Effect of Diet							
Control	4.15	1.11 [b]	3.00	691	112	51.5	11.0
Low-CP	3.91	1.53 [a]	2.39	700	115	52.2	10.9
Low-CPME	4.00	1.47 [a]	2.63	687	106	51.2	10.8
Effect of Type of Phytase							
FP	3.85	1.45	2.45	696	100	51.3	10.7
BP	3.87	1.42	2.46	690	102	51.1	10.6
Interaction Diet × Phytase							
Control	4.15	1.10	3.00	691	112	51.5	11.0
Low-CP × Uns	4.04	1.51	2.54	712	141	52.6	11.0
Low-CP × FP	4.08	1.59	2.49	698	106	53.0	10.9
Low-CP × BP	3.61	1.47	2.14	690	99.5	51.0	10.7
Low-CPME × Uns	4.24	1.53	2.71	677	120	52.7	11.3
Low-CPME × FP	3.63	1.47	2.43	693	94.4	49.6	10.7
Low-CPME × BP	4.14	1.38	2.76	689	104	51.2	10.4
SEM	38.7	0.059	0.212	8.101	6.70	8.20	0.270
***p* Values**							
Diet	NS	0.0001	NS	NS	NS	NS	NS
Phytase type	NS	NS	NS	NS	0.0001	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS

[a], [b] means within a column with different superscripts are significantly different ($p < 0.05$); FP—fungal phytase; BP— bacterial phytase; Uns—unsupplemented; SEM, standard error or mean; NS—not significant. Low-CP, low crude protein diet; low-CPME, low crude protein and metabolizable energy diet; TP—total protein; Alb—albumin; Glob—globulin; TL—total lipids; Chol—cholesterol; AP—alkaline phosphatase; AST—aspartate aminotransferase.

4. Discussion

4.1. Effect of Protein and Energy Level in the Diet

Reducing protein and energy levels in the diets did not affect the body weight gain and thus, the final weight of slow-growing broilers, showing that all the diets used in the trial were adequate to sustain the growth of colored broilers of Sasso strain from 1 to 64 d of age. This happened even if the feed, protein, and energy intake were reduced in both low-CP and low-CPME diets, and can be explained as the protein and energy conversion ratios were increased when CP and ME levels were reduced in the diets. Our results are in line with Khalifah [18], who found no significant differences on BW of slow-growing broilers when diets with 18, 16 or 14% CP were administered. Attia et al. [19] indicated that the protein effect is age-dependent since it has a significant impact on BW of chickens at 4 weeks of age, but no effects were observed later on. However, the increase in protein and energy conversion ratios is not tied to an increase of protein or metabolizable energy digestibility. Similarly, Attia et al. [20] and Shaldam [21] found no improvement in crude protein, ether extract, crude fiber, and ash digestibility due to decreasing protein and energy levels in broiler diets. Probably, the effect on nutrient digestibility is tied to the level of protein and/or energy reduction: in our trial, lowering 1 point percentage and 100 Kcal of protein and metabolizable energy, respectively, did not affect the digestibility coefficients of the nutrients. The decrease of nitrogen percentage in poultry excreta due to low-CP and low-PME diets (6.7%) is due to the reduction of ingested protein (5%). On the other hand, Abd-Elsamee [22] indicated that decreasing dietary crude protein significantly increased OM's digestibility coefficients, CP, EE, and nitrogen retention. It is not easy to explain the reduction of the intestine length recorded in poultry fed a low-CP diet, as no similar evidence is reported in the literature. Decreasing CP and CP + ME levels also caused an unexplained increase in plasma albumin without serum total protein changes. However, the most important result is that lowering CP and ME in diets reduced the feeding cost.

Considering that feeding cost is the highest cost in livestock production, this reduction also lowered the total cost of broiler production. Both reductions of energy and protein (4.1%) were more effective than the reduction of protein alone (2.5%). The decrease in feeding costs can be associated with either lower feed consumption, and/or lower prices of feed with lower nutritional value when acceptable performance was maintained.

4.2. Effect of Phytase Supplementation and Type

Otherwise, the diet, the effect of type of phytase in feed intake reduction (and thus in protein and energy intake reduction) can be explained due to the increase of nutrient digestibility (dry matter +3.8%; crude fiber +13.9%; crude protein +3.2%; ash retention +8.3%) and the decrease of nitrogen percentage in the feces (−12.1%). These results agree with Attia et al. [23], who found that phytase can improve the utilization of tropical native crops in laying hens. Besides, Ennis et al. [8] and Al-Harti et al. [24] found an improvement in nutrient digestibility and growth performance in broilers supplemented with microbial phytase. Even if the increase in nutrient digestibility was similar for both phytases, the impact on animal performance was different as bacterial phytase was more effective than fungal phytase in feed (5.5 vs. 3.2%), protein (7.2 vs. 4.9%), and energy (6.1 vs. 3.7%) intake reduction. The effect of phytase on CP digestibility could be attributed to an improvement in the amino acid's digestibility. Farrell et al. [25] indicated that phytase supplementation improved nitrogen retention by 2.7% and MEn by 2.3%, and this partly reflects the increase of DM and true ileal amino acid digestibility. Similar results were obtained by Attia et al. [1] and Rutherfurd et al. [26] when microbial phytase was supplemented to the diets. The surprising finding in this research was the positive effect of phytase on apparent digestibility of crude fiber. This could suggest further positive effects such as the increase of ME value of the diets [1,20,27]. Johnson et al. [28] observed an improvement of fiber digestibility when 4500 FTU of an *E. coli* phytase were supplemented to broiler diets. The different effects of type of phytase on nutrient intake could be ascribed to the different characteristics of the two enzymes. In fact, fungal and bacterial phytase have different optimal pH and resistance to pepsin which can affect the amount of digested and degraded phytase in the upper gastro-intestinal tract of poultry [29].

Several authors [15,30,31] showed that phytase supplementation to broiler, duck, and Japanese quail diets did not affect plasma protein, lipids, and cholesterol. In literature, the effect of phytase on tibia ash and mineral contents depends on the type of enzyme: In fact, *E. coli* phytase had a stronger effect than the fungal phytase-3 [32]. On the contrary, Jendza et al. [33] and Pillai et al. [34] observed that the source of phytase did not affect tibia ash. Along the same line, Payne et al. [35] and Veum et al. [36] did not observe differences between *E. coli* and P. Lycii phytase in bone-breaking strength, ash weight, and apparent absorption (g/d and %) of P, Ca, Mg, Zn, Fe, and Cu when used at 500 U. This probably happens because, although feed intake decreased due to the use of phytases, the enzymes supplied a higher amount of phosphorus and calcium as confirmed by their higher levels in blood. El-Deeb et al. [37] showed that broilers fed diets supplemented with phytase had serum inorganic phosphorus concentration similar than the positive control. Attia et al. [1] found that phytase supplementation increased plasma P of broilers fed diets with suboptimal levels of CP and ME. Perney et al. [38] reported that phytase supplementation to a maize-soybean meal diet containing less phosphorus than NRC's recommended level [11] increased tibia ash and plasma P of broilers. Rodehutscord and Pfeffer [39] found that blood serum phosphate, but not Ca, increased when phytase was supplemented to duck diets. The increase in plasma P observed in our trial can be ascribed to an increase in its digestibility: On this regard, Mireles-Arriaga et al. [40] have shown increased digestibility of total phosphorus from 35.81 to 15.87% due to the addition of phytase in broiler diets.

In our study, a few differences are between the two types of phytases; in particular, 6-*E. coli* phytase reduced feed intake, protein, and energy intake. Our results are according to Ptak et al. [41], who found that bacterial phytase from *E. coli* strongly reduced feed intake

than phytase from *A. niger* than the control. Our results can be explained as the 6-phytase needs to deplete a greater proportion of the phytate pool for equivalent phosphorus release compared to 3-phytase [10]; therefore, the energy and amino acids' units obtained per unit of 6-phytase activity are larger than 3-phytases. This can induce a higher amount of nutrient availability and thus, a reduced feed intake. As a direct consequence of lowering feed intake, the use of phytase reduces the feeding and total costs with greater effects due to the use of bacterial phytase.

4.3. Effect of the Interaction Diet × Type of Phytase

Considering the number of criteria considered in our trial, just some of them were significantly affected by the interaction diet x type of phytase (feed, protein and energy intake, and abdominal fat), and this suggests that the two types of phytase, in general, act in the same way in both diets. This agrees with several authors, including Qota et al. [30] and Attia [20].

Mortality rate was not different among the experimental group, suggesting no effect on types of diet and/or phytase supplementation. The overall incidence of mortality 1% was in normal range for broilers [22,24,30]. It should be mentioned that the feed intake was corrected for differences in mortality among pens and treatments, and thus the FCR was based corrected/actual feed consumption among the experimental treatments. However, there were still in-avoided cases which cannot be corrected, such as variation in space allowance and thus bird's activity, and it has an effect on energy expenditure and increasing feeding space in the pens.

5. Conclusions

The use of phytase, independently from its source, allows reducing the protein and energy content of Ca and P adequate diets for colored broilers of Sasso strain during 1–64 days of age. The use of 6-*E. coli* phytase induced the lowest feed intake without adverse effects on the body weight gain of broiler and improvements of feed conversion ratio. The supplementation of *A. niger* increased abdominal fat deposition of compared low-CPME diet compared to low-CPME_uns diet. All diets showed similar production index allowing the use of low-CPME diet when phytases were supplemented. In addition, the decrease of the nitrogen content in the feces could be a very important finding concerning the reduction of the environmental burden.

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