Effects of feeding diets containing heated hemp seed cake and phytase enzyme on the bone mineral of broiler chickens

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Abstract. The purpose of the study was to investigate the impact of heated hemp seed cake and phytase enzyme inclusion into the diet on the mineral content of the tibia and femur in broiler chickens. Unsexed Ross broiler chicks (210 birds) were randomly allocated into 7 treatments and 6 replicates and reared for 42 days. The treatments were P1= diet based on corn-soybean base; P2= diet containing 15% of HSC; P3= P2 with 1000 FTU/kg phytase enzyme; P4: diet containing 15% of autoclaved HSC; P5= P4 with 1000 FTU/kg phytase enzyme; P6= diet containing 15% of oven-heated HSC; P7= P6 with 1000 FTU/kg phytase enzyme. The findings indicated that the inclusion of HCS in the diet resulted in enhancement ($P<0.05$) in the calcium content of the tibia. Dietary autoclaved HSC with phytase enzyme significantly increased ($P<0.05$) phosphorus and potassium of the tibia compared to soybean and raw HSC-based diet. However, the treatments had no impact ($P>0.05$) on the magnesium, and calcium to phosphorus ratio (Ca:P) of tibia. In the femur bone, dietary autoclaved HSC with phytase enzyme significantly improved ($P<0.05$) calcium compared to a diet based on soybean meal, raw HSC, and oven-heated HSC without phytase enzyme. Dietary autoclaved HSC with phytase enzyme significantly improved ($P<0.05$) phosphorus of the femur compared to soybean meal and autoclaved HSC-based diet and increased ($P<0.05$) potassium compared to soybean meal, raw HSC-based diet. The treatments did not significantly affect ($P>0.05$) the magnesium levels in the femur. It is concluded the dietary autoclaved HSC with phytase enzyme is effective in improving the calcium, phosphorus, and potassium of the tibia and femur bone of the broiler.

1 Introduction

Bone health and strength in broiler chickens play an important role, especially in supporting optimal production performance. Bone quality is highly dependent on the intake of essential minerals such as calcium and phosphorus, which are generally obtained through feed [1]. However, mineral absorption from feed is often hindered by the presence of anti-nutritional

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compounds such as phytate [2]. This condition emphasizes the importance of feed innovations that not only meet the nutritional needs of chickens but also increase mineral bioavailability to support optimal bone health.

Hemp seed cake (HSC) is becoming one of the alternative feed ingredients attracting attention in poultry feed formulation in Europe countries. HSC, a by-product of hemp seed oil extraction, is abundant in protein (45%), minerals, and various other nutrients, making it a potential feed source [2]. However, the content of anti-nutrient compounds such as phytate, tannin, and saponin [2,3] in HSC can reduce mineral absorption by the chicken body ultimately affecting bone quality. Heat treatment is a traditional physical method used to decrease the antinutrients. In addition, heating improves the palatability and digestibility of carbohydrates in feeds [4]. Previous studies have reported that a temperature of 120^0C reduced antinutrients and improved protein digestibility. Heat-treated hemp seed (120^0C; 60 min) resulted in more palatable than raw hemp seed [4]. Meanwhile, the addition of phytase enzymes can help break down phytate and increase the availability of essential minerals and feed efficiency [5].

The combination of heated HSC and the addition of phytase enzymes is expected to be a promising strategy to maximize the nutritional potential of HSC as a poultry feed ingredient. By reducing anti-nutrient content and increasing mineral availability, it is expected that broiler bone health can be well maintained, thus supporting optimal growth and reducing the risk of fractures or other skeletal problems that often occur in fast-growing broilers. Therefore, the purpose of our research was to investigate the impact of using heated HCS with the addition of phytase enzyme on the mineral content (Ca,P,Mg,K) of the tibia and femur.This study will provide information on the impact of heating and adding phytase enzyme in the diet on anti-nutrients of HSC and bone mineral deposition of broiler chickens.

2 Materials and methods

2.1 Research duration and location

Our study was carried out at the Laboratory of Poultry Nutrition and Black Sea Advanced Technology Research and Application Center, Ondokuz Mayis University, Turkiye from March to August 2023.

2.2 Preparation of HSC

HSC was obtained by cold pressing local Nar Sarayı hemp seeds. Then, HSC in pellet form was floured before being oven-dried (120°C;20 minutes) or autoclaved (120°C;10 minutes). Heated and unheated HSC were analyzed for antinutrients (tannins, HCN, phytic acid, saponins) by the method of [6].

2.3 Animals and treatments

The chickens were reared under the approval of the Ethics Committee of Ondokuz Mayis University (No: 57/2022). A total of 210 Ross strain broiler chicks were housed in 42-floor cages with 1x1.30 m partition cages for 42 days. The diet was formulated based on corn, soybean meal, and HSC, following the nutrient specifications recommended for the Ross strain (Table 1). The study employed a completely randomized design (CRD) comprising 7 treatments with 6 replications. The treatments were:

P1= diet based on corn-soybean base;
P2= diet containing 15% of HSC
P3= P2 + 1000 FTU/kg phytase enzyme
P4= diet containing 15% of autoclaved HSC
P5= P4 +1000 FTU/kg phytase enzyme
P6= diet containing 15% of oven-heated HSC
P7= P6 +1000 FTU/kg phytase enzyme.

The environmental temperature was set at 33°C in the first two days and reduced by 1°C per day to 20°C during the finishing phase. The lighting system was supplied for 24 hours during the first three days to ensure that the chicks received sufficient light. After the third day, the lighting was changed to 23 hours of light and 1 hour of dark to support optimal growth while providing rest time for the chicks. The Gumboro vaccine was administered on day 21, while the Newcastle vaccine was administered on day 28.

Table 1. Ration composition and nutrient content.

Ingredients (%)	Starter		Grower		Finisher	
	Control	HSC	Control	HSC	Control	HSC
Yellow corn	53.2	48.31	59	54	65.18	60.2
Soybean meal	39.5	29	34.5	24	29	18.5
HSC	-	15	-	15	-	15
Vegetable oil	3.15	4	3.2	4	2.8	3.6
DCP	2.17	2.12	1.76	1.68	1.42	1.39
Limestone	0.8	0.8	0.58	0.6	0.56	0.56
Salt	0.37	0.37	0.37	0.37	0.37	0.37
L-Threonine	0.18	0.01	0.04	0.01	0.04	0.01
DL-Methionine	0.31	0.27	0.27	0.23	0.26	0.22
L-Lysine	0.22	0.02	0.18	0.01	0.27	0.05
Premix	0.1	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100	100
Nutrients						
CP (%)	23.5	23.5	21.5	21.6	19.5	19.5
ME (kcal/kg)	2982	2981	3061	3058	3112	3107
Extract ether (%)	2.60	4.01	2.80	4.30	2.96	5.06
Lysine (%)	1.32	1.32	1.18	1.19	1.1	1.08
Ca (%)	0.95	0.95	0.78	0.76	0.65	0.66
Av. phosphorus (%)	0.52	0.53	0.43	0.42	0.36	0.36

2.4 Sample analysis

On day 42, 2 chickens from each replicate were slaughtered for tibia and femur mineralization analysis. The left tibia and femur bones were taken and cleaned of muscle tissue. The tibia and femur were dried (105°C;24 h), then heated (600°C;48 h) to measure

ash content, followed by mineral analysis [7]. The mineral content (Ca, P, Mg, K) of bone was analyzed using a Scanning Electron Microscope (JSM-IT700HR) at the Black Sea Advanced Technology Research and Application Center, Ondokuz Mayiz University, Turkiye.

2.5 Statistical analysis

Analysis of Variance (ANOVA) was applied in data analysis with SPSS Statistics 22. $P< 0.05$ was declared significant and further tested using Duncan's Multiple Range Test.

3 Results and discussion

In our study, dietary HSC autoclaved with the addition of phytase enzyme was shown to increase calcium, phosphorus, and potassium content in the tibia and femur (Table 2). This positive effect was attributed to the effectiveness of autoclaving in reducing anti-nutrients. Heating treatment of HSC reduced the content of saponins, tannins, HCN, and phytic acid. Saponin content was lowest in oven-treated HSC (2.2 mg/g) compared to autoclaved (2.3 mg/g) and unheated HSC (3.2 mg/g) (Fig. 1). Similarly, the tannin content of HSC decreased slightly after autoclaving (2.93 mg tannat acid/g) compared to HSC without heating (3.57 mg tannat acid/g). HCN content showed the most significant decrease after autoclaving, with a level of 15.24 mg/kg compared to HSC without heating (22.66 mg/kg). This indicates that autoclaving is more effective than an oven in reducing antinutrients, thus improving HSC quality as a feed ingredient. This decrease in anti-nutrients is favorable for broiler chickens. Previously, a high dietary tannin level of 0.56% lowered aminopeptidase, and growth of broiler chickens [8]. Saponins and tannins are capable of complex binding with proteins, and digestive enzymes and reduce gut health [6]. Tannins and saponins suppress growth due to their ability to reduce digestibility of protein [9].

Autoclaving resulted in an 8.29% reduction in phytic acid content, whereas oven heating achieved only a 1.11% reduction (Fig. 1). Autoclaving at 120°C for 10 minutes works on the principle of high-pressure steam, which probably allows deeper penetration of heat and degrades the structure of anti-nutrients, especially phytate [10]. Phytate is a complex compound that can bind to essential minerals such as calcium, magnesium, and phosphorus [11], thus inhibiting their absorption in the digestive tract of chickens. At 120°C, the structure of phytate may initiate breakdown, releasing trapped minerals and reducing its binding ability. In line with previous studies that autoclaving is the best treatment in reducing anti-nutrients (tannins, trypsin inhibitors) in Jack Bean compared to soaking and cooking [12] and drastically reducing phytic acid content in chickpea *(Cicer arietinum* L.) [13]. However, it should be noted that the heating process should be conducted carefully, as excessive heating may damage other important nutrients such as essential amino acids or heat-sensitive vitamins. For instance, protein and starch content also decreases when legumes are autoclaved for 18-90 minutes at 121^0C and 20 minutes at 128^0C [14].

On the other hand, phytase enzymes are enzymes that specifically function to break down phytate bonds, which are complexes of phytic acid and minerals. Phytate binds phosphorus in a form that cannot be digested by poultry as they do not have endogenous phytase in their digestive tract [5]. By adding phytase to the feed, this enzyme works along the chicken's digestive tract to hydrolyze the phytate bonds, releasing the stored phosphorus as well as other minerals such as calcium. Regarding the use of phytase enzymes, [10] stated

that Ca and P released through phytate hydrolysis increased the proliferation of lactic acid bacteria in broiler chickens. In addition, hydrolyzing phytate, minerals, and other nutrients that form complexes with phytate molecules can improve bone parameters and gut morphology [15].

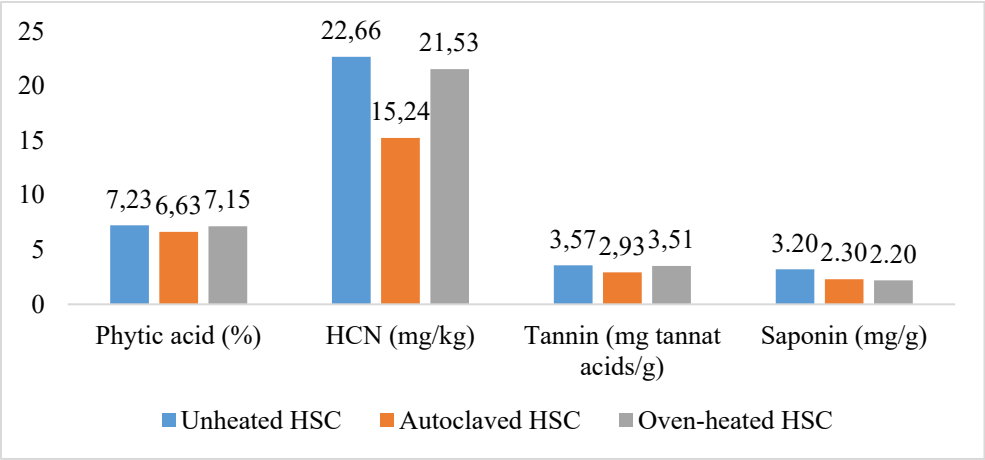

Fig.1. Effect of heating on HSC anti-nutrient content

Table 2. Effects of dietary HSC and phytic acid addition on the mineral content of tibia and femur of 42-day-old broiler chickens.

Para-meters	P1	P2	P3	P4	P5	P6	P7	SEM	P-Value
Tibia									
Ca	36.40^{a}	38.17^{b}	39.11^{b}	38.81^{b}	38.94^{b}	38.77^{b}	38.14^{b}	0.02	0.03
P	16.59^{a}	16.61^{ab}	16.82^{abc}	16.52^{a}	17.29^{c}	17.12^{bc}	17.02^{abc}	0.08	0.02
Mg	0.71	0.78	0.77	0.71	0.66	0.67	0.75	0.01	0.15
K	0.76^{a}	0.72^{a}	0.97^{ab}	0.94^{ab}	1.24^{b}	1.31^{b}	1.32^{b}	0.06	0.03
Ca:P	2.19	2.30	2.33	2.35	2.25	2.27	2.24	0.02	0.31
Femur									
Ca	36.56^{a}	37.49^{abc}	37.37^{ab}	38.38^{bcd}	39.48^{d}	37.65^{abc}	38.73^{cd}	0.18	<0.01
P	16.31^{a}	16.84^{ab}	16.73^{ab}	16.39^{a}	17.38^{b}	17.16^{b}	17.01^{ab}	0.10	0.02
Mg	0.61	0.72	0.75	0.64	0.73	0.72	0.73	0.02	0.18
K	0.99^{a}	1.31^{a}	1.15^{a}	1.83^{b}	1.86^{b}	1.39^{ab}	1.49^{ab}	0.08	<0.01
Ca:P	2.24^{a}	2.23^{a}	2.23^{a}	2.41^{b}	2.21^{a}	2.19^{a}	2.27^{ab}	0.02	<0.01

4 Conclusion

Dietary autoclaved HSC at 120°C for 10 minutes with phytase enzyme (1000 FTU/kg) is effective in improving the calcium, phosphorus, and potassium of the tibia and femur bone of the broiler.

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