

# Regression-derived phosphorus digestibility responses of broiler chickens to heat treatment of soybean meal and poultry meal

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**ABSTRACT** Two studies were conducted to evaluate the effects of autoclaving soybean meal (**SBM**) or poultry meal (**PM**) on their respective regression-derived phosphorus (**P**) ileal digestibility and utilization coefficients. On day 19 post hatching 384 or 320 Cobb 500 male broiler chickens were individually weighed and allotted into 6 or 5 treatments in Experiment 1 or 2, respectively. Both experiments consisted of 8 replicate cages with 8 birds per cage in a randomized complete block design. In the first study, 6 diets were formulated with either non-autoclaved or autoclaved soybean meal at 380, 480, or 580 g/kg in a 2 × 3 factorial. The second consisted of 5 diets including one corn-soybean meal and cornstarch based basal diet and 4 diets with 40 or 80 g/kg of non-autoclaved PM or autoclaved PM (**APM**). Chromic oxide was added as an indigestible index to determine the ileal digestibility and retention of nutrients. Birds received the experimental diets for 3 d and excreta collection was conducted during the

last 2 d. At the end of the experiments all birds were euthanized by CO<sub>2</sub> asphyxiation and ileal digesta samples were collected. Data were analyzed by ANOVA using the GLM procedure. In both studies autoclaving decreased ( $P < 0.05$ ) DM digestibility and retention. Increasing the inclusion level of test ingredients caused a linear increase ( $P < 0.05$ ) in intake of digestible and utilizable P in both studies, and linear reductions in the digestibility and retention of DM and P in the soybean meal study. Inclusion of autoclaved SBM resulted in higher ( $P < 0.01$ ) ileal digestibility of P and retention of P and Ca. The estimated ileal digestibility of P in SBM, autoclaved SBM, PM and APM were 45, 53.6, 61.2, and 61.2%, respectively, the corresponding retention were 40.6, 45, 51.7, and 59.2%, respectively. Comparison of the regression coefficients revealed that autoclaved SBM tended ( $P = 0.058$ ) to have higher P digestibility than non-autoclaved while no effect was noted with PM.

**Key words:** broiler, digestibility, heat treatment, phosphorus, soybean meal

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## INTRODUCTION

In the livestock industry, heat treatment is used to improve the digestibility of some ingredients by reducing the concentration of antinutritional factors, such as trypsin inhibitors in soybean meal (**SBM**) or glucosinolates in canola meal (González-Vega et al., 2011; Almeida et al., 2014). In the commercial setting, soybean meal is treated to temperatures between 100 and 110°C with steam during desolventizing and then with hot air that can reach 150°C until it reaches the optimal moisture content for proper conservation (Oliveira et al., 2020).

However, increasing the temperature and duration of treatment of feedstuff produces a negative effect on the

digestibility of energy and nutrients. Increasing autoclaving time and temperature of soybean meal or canola meal (ranging from 125 to 150°C and for 3–30 min) linearly decreased amino acid digestibility and metabolizable energy (**ME**) for pigs (González-Vega et al., 2011; Almeida et al., 2014; Oliveira et al., 2020). The detrimental effect of heat damage on amino acid digestibility is mainly due to Maillard reactions, which have also been linked to lower metabolizable energy in pigs and chickens (Oliveira et al., 2020; Sung et al., 2022).

However, some studies have indicated that treatments such as soaking, fermenting or heating have the opposite effect on P, increasing the bioavailability and digestibility. Improvements in bioavailability of P due to heat treatment were reported in chicks and pigs (Amezcuca et al., 2004; Amezcuca and Parsons, 2007). These may be due to a reduction in the phytic acid P, which is similar to observations when legumes were extruded (El-Hady and Habiba, 2003).

Poultry meal (**PM**) is produced from the non-edible parts of chicken meat processing, notably skin, head, viscera

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and bones. The composition of these products may vary with the amount of each component, generally it results in a high calcium and protein content together with highly digestible P (Rojas and Stein, 2013; Park et al., 2020; Adekoya et al., 2021). Due to its animal origin, poultry meal contains a negligible amount of phytate P, whereas this is the main P reserve in plants (Schlemmer et al., 2009). Phytate (myo-Inositol hexaphosphate (**InsP6**)) has a strong negative charge under pH 6 to 7 and can chelate with positively charged compounds, for example, Ca, Fe, Mg, and Zn. It remains quite stable to temperatures of up to 100° C (Schlemmer et al., 2009). Phytate is accountable for reducing the digestibility of other nutrients in monogastric animals due to its low solubility and capacity to form complexes with proteins and minerals, decreasing the nutritional value of the diet (Cowieson et al., 2017; Zhang and Adeola, 2017). Because broilers have low endogenous phytase activity, most of the phytate bound P remains undigested and is excreted, thus contributing to feed wastage and environmental pollution (Humer et al., 2015).

Providing adequate levels of nonphytate P in the diet enables an improvement in feed utilization and bird performance while mitigating the environmental impact. Therefore, it is of interest to assess the effect of heat treatment on the degradation of phytate and hence on the digestibility of P for broiler chickens. Previous studies indicate that autoclaving may improve P digestibility for broiler chickens. However, to the best of our knowledge no study has been conducted to investigate this hypothesis. Therefore, 2 experiments were conducted to determine the impact of heat treatment on P digestibility of 2 distinct ingredients for broiler chickens by determining the ileal P digestibility and apparent total tract utilization of P using the regression method.

## MATERIALS AND METHODS

All protocols used in the study were approved by the Purdue University Animal Care and Use Committee (West Lafayette, IN).

### **Autoclaving Method**

Part of the soybean meal and the poultry meal were autoclaved using a Beta Star Life Science Equipment autoclave model LS 243648-SRM-HHC-LNCT-LNL (R-V Industries, Inc., 584 Popular Road Honey Brook, PA). The following autoclaving conditions were used: jacket temperature, 134°C; purge time, 6 min; purge/prevacuum pressure, 150 kPa; number of pulses, 4; prevacuum pressure, 25 kPa; prevacuum hold time, 3 min; team charge stabilize time, 1 min; sterilization temperature, 134°C; dry cycle time, (40 min for soybean meal and 120 min for poultry meal); and dry cycle vacuum pressure, 90 kPa.

### **Birds, Management, and Sample Collection**

Male broiler chicks (Cobb 500; Cobb-Vantress Inc., Siloam Springs, AR) were housed in electrically heated

battery brooders (model SB 4 T, Alternative Design Manufacturing, Siloam Springs, AR) on day 0 post hatching. Temperature and lighting were maintained as previously described (Haetinger et al., 2021). Birds received a standard mash starter diet formulated to meet or exceed nutrient requirements recommended by NRC (1994) before the initiation of experiments. Feed and water were provided ad libitum throughout the experiment. On day 19 post hatching, birds were individually weighed, 384 were allotted to 6 treatments in Experiment (**Exp.**) 1 and 320 birds were allocated to 5 treatments in Exp. 2. Both experiments were comprised of 8 replicate cages per treatment with 8 birds per cage in a randomized complete block design with body weight as a blocking factor.

Experimental diets were provided for 3 d. Excreta collection was conducted for last 2 d of feeding experimental diets by lining collection pans with waxed paper. After 3 d of feeding experimental diets, all birds were euthanized by CO<sub>2</sub> asphyxiation, weighed individually, and dissected to excise the ileum, which was estimated as the portion of distal small intestine from the Meckel's diverticulum to ileocecal junction. Ileal digesta samples were collected from distal two-thirds of the ileum 2 cm proximal to the ileocecal junction by flushing contents with distilled water. Collected ileal digesta samples were pooled within cages and immediately stored at -20°C. Body weight gain (**BWG**) and feed intake (**FI**) (g/bird) during the experimental periods were recorded and gain to feed ratio (**G:F**) (g/kg) of each cage was calculated.

### **Test Ingredients and Experimental Diets**

Soybean meal in Exp. 1 and poultry meal in Exp. 2 were autoclaved at 134°C for 40 and 120 min, respectively. Semipurified test diets were formulated maintaining the Ca:P ratio at approximately 1.4:1. A 2 × 3 factorial arrangement was used in Exp. 1, with 2 test ingredients (non-autoclaved SBM or autoclaved SBM) and 3 inclusion levels (380, 480, or 580 g/kg). In Exp. 2 a corn-soybean meal and cornstarch-based basal diet was formulated along with other 4 diets in which each of non-autoclaved or autoclaved poultry meal was supplemented at 40 or 80 g/kg. In both experiments chromic oxide was added as an indigestible index marker at 5 g/kg of diet in the form of a premix (Tables 1 and 2).

### **Chemical Analysis**

Excreta and ileal digesta samples were placed in a forced-air drying oven at 55°C until constant weight. Feed ingredients, experimental diets, excreta, and ileal digesta samples were ground (<0.75 mm) using a centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany). Ground ingredients, experimental diets, excreta, and ileal digesta samples were analyzed for DM by drying at 105°C overnight in a forced-air drying oven (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Diets, ileal digesta and excreta samples were

**Table 1.** Ingredient and calculated nutrient composition in experimental diets for Experiment 1 fed to broiler chickens.

Ingredient, g/kg	Intact soybean meal, g/kg			Autoclaved soybean meal, g/kg		
	380	480	580	380	480	580
Dextrose	100.0	100.0	100.0	100.0	100.0	100.0
Soybean meal, 48% CP	380.0	480.0	580.0	0.0	0.0	0.0
Autoclaved soybean meal	0.0	0.0	0.0	380.0	480.0	580.0
Cornstarch	408.5	306.6	204.8	408.5	306.6	204.8
Dried egg albumen	20.0	20.0	20.0	20.0	20.0	20.0
Soybean oil	50.0	50.0	50.0	50.0	50.0	50.0
Ground limestone	7.0	8.9	10.7	7.0	8.9	10.7
Salt	4.0	4.0	4.0	4.0	4.0	4.0
DL-Methionine	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin-mineral premix <sup>1</sup>	3.0	3.0	3.0	3.0	3.0	3.0
Chromic oxide premix <sup>2</sup>	25.0	25.0	25.0	25.0	25.0	25.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Analyzed nutrient, g/kg as fed basis						
DM	879.4	865.5	879.1	881.0	871.7	884.1
Crude protein	198.6	243.9	295.2	192.5	243.2	290.5
P	2.6	3.4	4.0	2.6	3.3	4.0
Ca	3.7	4.5	5.6	3.8	4.5	5.7
Calculated nutrient						
MEn, kcal/kg	3,198	3,070	2,942	3,198	3,070	2,942
Non-phytate P	1.1	1.3	1.6	1.1	1.3	1.6

<sup>1</sup>Provided the following quantities per kg of complete diet: vitamin A, 5,145 IU; vitamin D3, 2,580 IU; vitamin E, 17.15 IU; menadione, 4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11.0 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 0.01 mg; biotin, 0.06 mg; thiamine mononitrate, 2.20 mg; folic acid, 0.99 mg; pyridoxine hydrochloride, 3.30 mg; I, 1.11 mg; Mn, 107 mg; Cu, 4.44 mg; Fe, 73.5 mg; Zn, 179 mg; Se, 0.43 mg.

<sup>2</sup>Prepared as 1 g chromic oxide added to 4 g cornstarch.

analyzed for chromium concentration using a spectrophotometer (Spark 10 M; Tecan Group Ltd., Männedorf, Switzerland) after a wet-ash digestion as described by Fenton and Fenton (1979). Concentration of P in samples was determined from digested samples, by spectrophotometry, with absorbance read at 630 nm. Calcium concentration in samples was determined by flame atomic absorption spectrometry using Varian Spectr.AA 220FS (Varian Australia Pty Ltd., Victoria, Australia).

### Calculations and Statistical Analysis

The apparent ileal digestibility (AID) or apparent total tract utilization (ATTU) of P, Ca and DM in ileal digesta and excreta were calculated using the index method (Kong and Adeola, 2014).

$$AID \text{ or } ATTU \text{ of } P (\%) = 100 - \left[ \left( \frac{Cr_i \times P_o}{Cr_o \times P_i} \right) \times 100 \right]$$

**Table 2.** Ingredient and calculated nutrient composition in experimental diets for Experiment 2 fed to broiler chickens.

Ingredient, g/kg	0	Intact poultry meal, g/kg		Autoclaved poultry meal, g/kg	
		40	80	40	80
Ground corn	510.0	510.0	510.0	510.0	510.0
Soybean meal, 48% CP	230.0	230.0	230.0	230.0	230.0
Poultry meal	0.0	40.0	80.0	0.0	0.0
Autoclaved poultry meal	0.0	0.0	0.0	40.0	80.0
Cornstarch	135.3	95.4	55.5	95.4	55.5
Dried egg albumen	60.0	60.0	60.0	60.0	60.0
Soybean oil	25.0	25.0	25.0	25.0	25.0
Ground limestone	6.7	6.6	6.5	6.6	6.5
Salt	4.0	4.0	4.0	4.0	4.0
DL-Methionine	1.0	1.0	1.0	1.0	1.0
Vitamin-mineral premix <sup>1</sup>	3.0	3.0	3.0	3.0	3.0
Chromic oxide premix <sup>2</sup>	25.0	25.0	25.0	25.0	25.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Analyzed nutrient, g/kg as fed basis					
DM	878.4	872.5	882.2	883.7	886.6
Crude protein	192	215	256	217	248
P	2.7	3.6	4.2	3.4	4.2
Ca	3.5	4.8	6.0	4.8	5.9
Calculated nutrient					
MEn, kcal/kg	3253	3241	3229	3241	3229
Non-phytate P	0.9	1.5	2.1	1.5	2.1

<sup>1</sup>Provided the following quantities per kg of complete diet: vitamin A, 5,145 IU; vitamin D3, 2,580 IU; vitamin E, 17.15 IU; menadione, 4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11.0 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 0.01 mg; biotin, 0.06 mg; thiamine mononitrate, 2.20 mg; folic acid, 0.99 mg; pyridoxine hydrochloride, 3.30 mg; I, 1.11 mg; Mn, 107 mg; Cu, 4.44 mg; Fe, 73.5 mg; Zn, 179 mg; Se, 0.43 mg.

<sup>2</sup>Prepared as 1 g chromic oxide added to 4 g cornstarch.

where  $Cr_i$  is the chromium concentration in the diets and  $Cr_o$  is the concentration of chromium in ileal digesta or excreta;  $P_i$  is the P, Ca or DM concentration in the diets and  $P_o$  is P, Ca or DM concentration in ileal digesta or excreta.

The effect of autoclave, inclusion levels of test ingredients and their interaction were evaluated using a two-way analysis of variance in SAS. A  $2 \times 3$  factorial arrangement of 2 test ingredients (autoclaved or non-autoclaved) and 3 levels of soybean meal inclusion was used in data analysis for Exp. 1 data. The data analysis used in Exp. 2 was a  $2 \times 2 + 1$  of 2 test ingredients (autoclaved or non-autoclaved), 2 levels of inclusion plus the basal diet. Significance was set as  $P < 0.05$  and a trend was considered when  $0.05 < P < 0.1$ .

Orthogonal polynomial contrasts were conducted to determine the linear effects of increasing levels of test ingredients. Regression analysis between the test ingredient-associated apparent ileal digestible P intake or total tract retained P intake in g/bird and the P intake in g/bird was conducted using multiple linear regressions as described by Bolarinwa and Adeola (2012). Each test ingredient regression analysis consisted of 3 levels of test with 8 replicates per test ingredient level for a total of 24 data points.

Regression coefficients were compared as previously described (Adeola and Ilejeji, 2009). Because each level of test ingredients had 2 cages in each block of each experiment (autoclaved or non-autoclaved) and 8 blocks per test ingredient treatment, apparent ileal digestible P intake or total tract retained P intake in g/bird was regressed with P intake in g/bird for each block to generate intercepts and slopes for each of the 8 blocks per diet type (autoclaved or non-autoclaved). The intercept and slope data were analyzed as a one-way ANOVA in a completely randomized design using intercept or slope

as the dependent variable and diet type as the independent variable with 1 degree of freedom for diet type and 14 degrees of freedom for the error term. In this analysis, a block of 6 cages in Exp. 1 or 5 cages in Exp. 2 served as the experimental unit.

## RESULTS

Growth performance and digestibility results of Exp. 1 and 2 are presented in Tables 3 and 4, respectively. Comparisons of the regression coefficients between non-autoclaved and autoclaved ingredients are presented in Table 5.

### Soybean Meal

Inclusion of autoclaved soybean meal in diets increased the AID of P, ATTU of P and Ca, as well as decreased the AID of DM and Ca and ATTU of DM (Table 3). Gradually increasing the inclusion level of the test ingredients from 380 to 580 g/kg caused a linear increase in final BW, BW gain, feed intake, G:F, and produced linear decreases in ileal digestibility and ATTU of DM, P and Ca, except for AID and ATTU of Ca in non-autoclaved SBM diets. Additionally, an interaction between autoclave and linear contrast was found for the ATTU of Ca, which decreased linearly only with autoclaved SBM. Intake of digestible and utilizable P increased linearly with higher inclusion rates of test ingredients and diets with autoclaved soybean meal had higher digestible P intake.

The regression-derived ileal digestibility of P in soybean meal was 45% and in autoclaved SBM was 53.6% (Table 5). Regression-derived total tract utilization of P was 40.6% and 45% in SBM and autoclaved SBM,

**Table 3.** Growth performance, apparent ileal digestibility and apparent total tract utilization of DM, P, and Ca in experimental diets containing non-autoclaved or autoclaved (A) soybean meal (SBM) at 3 inclusion levels (IL) fed to broiler chickens in Experiment 1.

Item	Non-autoclaved SBM			Autoclaved SBM			SEM	P-values			Linear contrasts P-values	
	380 g/kg	480 g/kg	580 g/kg	380 g/kg	480 g/kg	580 g/kg		A	IL	A * IL <sup>1</sup>	Non-autoclaved SBM	Autoclaved SBM
Growth performance												
Initial BW, g	794	794	794	794	794	793	0.4	0.394	0.640	0.966	0.507	0.685
Final BW, g	940	965	991	824	844	852	7.5	<0.001	<0.001	0.274	<0.001	0.012
BW gain, g/bird	146	172	198	30	51	59	7.7	<0.001	<0.001	0.290	<0.001	0.013
Feed intake, g/bird	261	277	280	220	244	255	5.1	<0.001	<0.001	0.325	0.011	<0.001
Gain to feed ratio, g/kg	559	619	709	134	211	232	26.9	<0.001	<0.001	0.407	<0.001	0.015
Apparent ileal digestibility, %												
DM	82.8	78.4	75.3	69.8	65.1	58.7	0.93	<0.001	<0.001	0.120	<0.001	<0.001
P	54.0	53.1	49.9	69.7	65.7	63.0	1.52	<0.001	0.005	0.560	0.064	0.004
Ca	59.0	56.6	50.8	52.9	42.9	42.4	4.09	0.008	0.079	0.637	0.166	0.076
Apparent total tract utilization, %												
DM	80.1	75.6	70.6	67.5	62.9	58.7	0.91	<0.001	<0.001	0.870	<0.001	<0.001
P	53.7	54.0	49.5	60.5	57.4	54.6	1.89	0.002	0.031	0.675	0.127	0.032
Ca	15.8	14.7	16.1	61.8	55.3	48.2	2.37	<0.001	0.029	0.019	0.909	<0.001
Ileal digestible P intake, g/bird	0.36	0.49	0.56	0.40	0.53	0.64	0.018	<0.001	<0.001	0.412	<0.001	<0.001
Utilizable P intake, g/bird	0.35	0.50	0.55	0.35	0.46	0.55	0.021	0.443	<0.001	0.595	<0.001	<0.001

<sup>1</sup>Interaction of autoclave (A) and inclusion level (IL).

**Table 4.** Growth performance, apparent ileal digestibility and apparent total tract utilization of DM, P, and Ca in experimental diets non-autoclaved poultry meal (PM) or autoclaved (A) poultry meal (APM) at 2 inclusion levels (IL) fed to broiler chickens in Experiment 2.

Item	Basal	PM		APM		SEM	<i>P</i> -values			Linear contrasts <i>P</i> -values	
		40 g/kg	80 g/kg	40 g/kg	80 g/kg		Autoclave	IL	A * IL <sup>1</sup>	PM	APM
Growth performance											
Initial BW, g	794	795	794	794	794	0.2	0.174	0.417	0.754	0.402	0.929
Final BW, g	935	974	987	977	980	9.0	0.840	0.377	0.555	<0.001	0.002
BW gain, g/bird	141	179	193	183	186	9.0	0.869	0.363	0.547	<0.001	0.002
Feed intake, g/bird	263	271	281	287	281	7.2	0.290	0.749	0.290	0.091	0.091
Gain to feed ratio, g/kg	532	662	683	635	660	20.7	0.231	0.276	0.924	<0.001	<0.001
Apparent ileal digestibility, %											
DM	78.3	78.0	75.1	75.6	73.7	0.53	0.001	<0.001	0.312	<0.001	<0.001
P	48.0	54.6	54.4	45.8	54.2	1.29	0.002	0.003	0.003	0.002	0.002
Ca	58.3	66.8	65.5	61.9	63.7	2.56	0.201	0.928	0.550	0.056	0.146
Apparent total tract utilization, %											
DM	78.9	79.5	74.8	75.5	75.6	0.71	0.036	0.003	0.002	<0.001	0.003
P	44.9	51.2	49.3	44.2	52.6	1.43	0.225	0.032	0.001	0.039	0.001
Ca	36.6	43.9	42.5	44.2	52.1	3.17	0.130	0.316	0.155	0.196	0.002
Ileal digestible P intake, g/bird	0.34	0.53	0.65	0.45	0.64	0.018	0.017	<0.001	0.084	<0.001	<0.001
Utilizable P intake, g/bird	0.32	0.50	0.59	0.44	0.62	0.020	0.445	<0.001	0.031	<0.001	<0.001

<sup>1</sup>Interaction of autoclave (A) and inclusion level (IL).

respectively. Comparison of the regression coefficients for AID or ATTU of intact and autoclaved soybean resulted in a trend ( $P = 0.058$ ) when comparing the slopes of AID and was not significant for the intercepts or the ATTU coefficients (Table 5).

## Poultry Meal

Autoclaving poultry meal had no significant effect on final BW, BWG, feed intake or G:F, additionally, these parameters increased linearly with increasing levels of test ingredients, except for feed intake, where a trend was noted ( $P = 0.091$ ; Table 4).

**Table 5.** Comparison of regression-derived coefficients of ileal digestibility (RDID) and total tract utilization (RDTTU) of P in non-autoclaved and autoclaved soybean meal fed to broiler chickens in Experiment 1 and in non-autoclaved and autoclaved poultry meal fed to broiler chickens in Experiment 2.

Item	Intercept	Slope
Experiment 1		
RDID of P		
Non-autoclaved soybean meal	0.0633	0.4502
Autoclaved soybean meal	0.0954	0.5357
SEM	0.0280	0.0357
<i>P</i> -value	0.6528	0.0579
RDTTU of P		
Non-autoclaved soybean meal	0.1022	0.4064
Autoclaved soybean meal	0.0957	0.4496
SEM	0.0439	0.0589
<i>P</i> -value	0.95	0.6243
Experiment 2		
RDID of P		
Non-autoclaved poultry meal	-0.0804	0.6124
Autoclaved poultry meal	-0.1091	0.6123
SEM	0.0519	0.0376
<i>P</i> -value	0.393	0.8591
RDTTU of P		
Non-autoclaved poultry meal	-0.0288	0.5166
Autoclaved poultry meal	-0.1098	0.5924
SEM	0.0487	0.0461
<i>P</i> -value	0.2244	0.2285

Autoclaving decreased the AID of P and DM as well as ATTU of DM. Gradually increasing the inclusion level of the test ingredients resulted in a linear decrease in the AID and ATTU of DM and P. The ATTU of Ca linearly increased in diets with autoclaved PM but not in diets with regular PM. The intake of digestible and utilizable P increased linearly with higher inclusion rates of the test ingredients, autoclave had a significant effect on digestible P intake.

The regression-derived ileal digestibility of P was 61.2% in PM and 61.2% in autoclaved PM and the total retention of P was 51.7% and 59.2% in PM and autoclaved PM, respectively (Table 5). Comparison of the determined AID and ATTU regression coefficients of PM and APM were not significant.

## DISCUSSION

Treating feedstuff to mitigate the effects of some anti-nutrients such as enzyme inhibitors and non-starch polysaccharides or to improve the availability of nutrients to livestock are tools that may be implemented to improve feed efficiency. Common examples include heat treatment, pelleting, extrusion or fermenting. Most of the P excreted by poultry originates from the phytate fraction of plant-based ingredients, which is largely inaccessible to broiler chickens. However, phytate may, in part, be degraded by heat treatment into lower inositol phosphates, which would enhance P digestibility and hamper the formation of phytate complexes with other nutrients. Previous studies have reported improvements in bioavailability of P for broiler chickens and in digestibility of P for pigs from autoclaving ingredients (Amezcu, 2004; Amezcu and Parsons, 2007; Lee and Nyachoti, 2021). Therefore, this study aimed at investigating the impact of autoclaving soybean meal and poultry meal on their P digestibility and total tract utilization for broiler chickens using the regression method.

Adding heat damaged ingredients to broiler diets has consistently caused negative effects on protein digestibility and bird performance (Shirley and Parsons, 2000; Amezcua and Parsons, 2007). The preceding observations are congruent with our results, as in both studies autoclaving caused a decline in DM digestibility and apparent total tract utilization compared to non-autoclaved diets, which may have contributed to poorer growth performance of birds in these treatments. Although the concentration of total amino acids was maintained equal across diets along with similar ME, it was clear from the darker color of the autoclaved SBM that there was some heat damage. Hence it is reasonable to consider that amino acid and energy digestibility decreased in the autoclaved diets due to heat damage.

Zanu et al. (2020) found that autoclaving meat and bone meal decreased crude protein digestibility. Parsons et al. (1992) reported lower digestibility of several amino acids, mainly Lysine and a decrease in bird performance that were intensified with increasing autoclaving time. Similarly, Shirley and Parsons (2000) and Amezcua and Parsons (2007) observed a reduction in amino acids concentration and digestibility due to autoclaving meat and bone meal and distillers dried grains with solubles. Furthermore, Sung et al. (2022) found linear reductions in nitrogen retention when increasing autoclaving time of poultry meal fed to broilers and pigs. This effect may be explained by the Maillard reactions caused by the heat treatment, forming early and late Maillard compounds, that are carbohydrate-amino acid complexes of low digestibility (Fernandez and Parsons, 1996; Amezcua and Parsons, 2007). Additionally, an increase in the neutral detergent fiber (NDF) fraction may have also played a role in lowering the digestibility and retention of DM, as was reported by Lee and Nyachoti (2021) with soybean expeller and canola meal autoclaved at 121°C for 60 min. Moreover, Almeida et al. (2014) also reported an increase in acid detergent fiber (ADF) and NDF fraction from autoclaving soybean meal and indicated that it may be because some of the products of Maillard reactions are analyzed as ADF and NDF. Recent studies have also reported a decrease in metabolizable energy (ME) due to autoclaving ingredients. Oliveira et al. (2020) and Sung et al. (2022) reported linear declines in ME for pigs and broiler chickens when autoclaving soybean meal or poultry meal for gradually increasing temperature or time. This may be on account of the reductions in utilization of nitrogen and oxidative stress from the ingestion of heat damaged protein.

Linear reductions in ileal digestibility and apparent total tract utilization of DM, P, and Ca in diets were observed in Exp. 1. The lower digestibility of SBM energy and protein compared to the other main ingredients in the diet, i.e. dextrose, cornstarch, soybean oil and dried egg albumen, were probably responsible for the reductions observed in the AID and ATTU of DM. The lower digestibility of P and Ca in SBM, that had increasing levels of inclusion was mainly responsible for the observed linear reduction in P and Ca digestibility of diets. In the second experiment, inclusion of either poultry meal or

autoclaved poultry meal linearly increased the digestibility and retention of P, probably due to its high concentration and digestibility of P compared to the basal diet that included only plant sources of P.

Feed intake increased with higher inclusion levels of the test ingredients, which may be explained by the respective increase in non-phytate P concentration (from 1.1 to 1.6 g/kg in Exp. 1 and 0.9 to 2.1 g/kg in Exp. 2) and increase in the intake of digestible and utilizable P. Aderibigbe et al. (2022) reported that non-phytate P level plays a key role in the regulation of voluntary feed intake of broiler chickens through regulation of gene expression of appetite regulators in the gut-brain axis. Furthermore, as autoclaving may have lowered the ME of the test ingredients, birds may have increased feed consumption to meet their ME requirements. Sung et al. (2022) reported an increase in feed intake accompanying a decrease in ME of the diet as autoclaving time of poultry meal increased.

While heat treatment decreased DM digestibility, the opposite occurred with P digestibility, which improved in diets with autoclaved SBM, and the comparison of the regression determined ileal digestibility of P also indicated that P digestibility of soybean meal tended to be higher ( $P = 0.058$ ) when it was autoclaved (53.6%) compared to non-autoclaved (45%). This is consistent with Amezcua and Parsons (2007), that fed corn distillers dried grains with solubles autoclaved for 80 min at 121°C to chickens and reported an increase in tibia ash and P bioavailability. Moreover, improvements in the bioavailability of P due to heat treatment were also outlined by Amezcua et al. (2004) for distillers dried grains with solubles. Further, Lee and Nyachoti (2021) found an increase in Ca and P digestibility for pigs fed autoclaved soybean expeller, canola meal or canola expeller compared to non-autoclaved diets with these ingredients.

However, the explanations for the improvements in P utilization are still unclear, as literature indicating some degradation of phytate is inconsistent. Lee and Nyachoti (2021) did not find any differences in the phytate concentration in autoclaved ingredients (soybean expeller, canola meal or canola expeller) and Edwards et al. (1999) did not find any changes in inositol phosphate 4, 5, or 6 in pelleted or extruded corn and soybean meal based diets. Alternatively, Pontoppidan et al. (2007) indicated that heat treatment from extrusion shifted some of the inositol phosphates from InsP6 to InsP5. El-Hady and Habiba (2003) reported reductions of phytic acid concentration along with tannins, phenols, and  $\alpha$ -amylase and trypsin inhibitors due to higher temperature of extrusion in soaked faba beans, chickpeas, pea seeds, and kidney beans. Pontoppidan et al. (2007) also observed higher degradation of InsP6 in cereals (corn and wheat) compared to oilseeds. The authors did not find any changes into lower inositol phosphates; however, the process of extrusion employs lower temperature and duration than the heat treatment used by Lee and Nyachoti (2021) and in the present study. Furthermore, it is important to note that P and inositol-P vary widely

among grains, cultivars, crop years and other factors regarding soil characteristics and irrigation (Cossa et al., 2000), which must be considered when comparing results.

Another factor that may have contributed to increasing P digestibility in autoclaved soybean meal is an increase in phytase activity from the grains, as Carlson and Poulsen (2003) reported when soaking and fermenting barley or wheat. The authors indicated that fermentation time and temperature during soaking increased the natural phytase activity. However, as Pontoppidan et al. (2007) pointed out the endogenous plant phytases are generally more active in cereals than in oilseeds and there may be some short activation of these enzymes during thermal treatment but the extent of their effect on soybean meal may not be significant to affect digestibility.

Interestingly, a decrease in P digestibility of diets was observed when poultry meal was autoclaved. It may be that autoclaving did not affect P digestibility in poultry meal because of the lack of an appreciable amount of phytate in poultry meal; however, because protein and ME digestibility decreased, this imbalance may have contributed for the observed decrease in P digestibility. Xue et al. (2016) demonstrated a relationship between protein and P digestibility. The authors reported that low protein level in diets limited the P digestibility and retention in broilers, attributing this to a decrease in growth and gene expression of sodium-phosphate cotransporter IIb (Na-Pi-IIb). As both lean tissue and bone development occur concurrently in growing animals and these processes require amino acids and P in adequate levels.

Autoclaving poultry meal had no effect on Ca digestibility or retention, but in the soybean meal study it decreased Ca digestibility and increased the ATTU of Ca. These results are inconsistent with previous findings of Zanu et al. (2020), that observed an increase in Ca digestibility for broilers on d 16 post hatching that were fed meat and bone meal that had been autoclaved at 128°C for 90 min. Lee and Nyachoti (2021) found an increase in ATTU of Ca in growing pigs fed autoclaved ingredients (soybean expeller, canola meal, or canola expeller) compared to non-autoclaved diets. Indicating that the heat treatment may reduce the formation of Ca-phytate complexes, hence increasing the amount of free Ca that is absorbed.

The P digestibility of soybean meal and poultry meal determined on this study were similar to those reported by Rostagno et al. (2017), however the coefficients of poultry meal were lower than the ones reported by (Adekoya et al., 2021). This difference may be due to variations in the qualities of the material used (including proportion, types, and characteristics of starting raw material) and processing conditions involving pressure of steam and temperature during agitation and drying (Hicks and Verbeek, 2016; Park et al., 2020).

In summary, this study investigated the effects of autoclaving soybean meal and poultry meal on digestibility and utilization of nutrients for broiler chickens and found that the digestibility and retention of dry

matter reduced when autoclaved ingredients replaced non-autoclaved ones and the digestibility of phosphorus of soybean meal may be improved by heat treatment but not that of poultry meal. These results indicate that heat treatment may have an effect on phytate degradation or activity of intrinsic plant phytase, which could mitigate the use of mineral supplements and phosphorus excretion, however, further research is needed to establish such effect.

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## DISCLOSURES

The authors declare no conflict of interest.

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