CATHARINA GIOVANA MIRABILE

TRUE ILEAL PHOSPHORUS DIGESTIBILITY OF SOYBEAN MEAL FOR MALE AND FEMALE BROILER CHICKENS

Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Arele Arlindo Calderano

Co-advisers: Gabriel Cipriano Rocha
Melissa Izabel Hannas
Horacio Santiago Rostagno

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Assent:

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Aos meus pais, Elisabete Pereira Mirabile e Luiz Ricardo Mirabile,
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por todo amor e apoio ao longo dessa jornada.

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"Tudo o que é seu encontrará uma maneira de chegar até você"
Chico Xavier

BIOGRAFIA

CATHARINA GIOVANA MIRABILE, filha de Elisabete Pereira Mirabile e Luiz Ricardo Mirabile, nasceu em 28 de outubro de 1994, em São Paulo, Capital.

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ABSTRACT

MIRABILE, Catharina Giovana, M.Sc., Universidade Federal de Viçosa, February, 2021. **True ileal phosphorus digestibility of soybean meal for male and female broiler chickens**. Adviser: Arele Arlindo Calderano. Co-advisers: Gabriel Cipriano Rocha, Melissa Izabel Hannas and Horacio Santiago Rostagno.

To determine the true ileal P digestibility coefficient of soybean meal (SBM), a total of 384 broilers (Cobb 500) at 21 days old, 192 males and 192 females, were arranged in a 3 x 2 (three total P levels x sex) factorial assay in a completely randomized design, with eight replicates and eight birds per experimental unit. The levels of total P in the experimental diets were 2.8, 3.5 and 4.2 g/kg. Increases in total P levels were achieved with the exclusive inclusion of SBM. Diets were provided from 21 until 26 days old, when the ileal digesta of the birds were collected. After chemical analyses, the ileal digestible P content of the diets was plotted against total P concentration in a linear regression analysis. The slope of the regression line gives the true ileal P digestibility coefficient of SBM. There was no interaction between sex and total P of diets ($P > 0.05$). There was linear increase in ileal P digestible content ($P < 0.001$) with increasing levels of total P in the diets, according to the equation $Y = 0.419X+2.350$. In conclusion, the true ileal P digestibility coefficient in SBM estimated for male and female broilers chickens was 0.419.

Keywords: Digestibility. Phosphorus. Poultry. Soybean meal.

RESUMO

MIRABILE, Catharina Giovana, M.Sc., Universidade Federal de Viçosa, fevereiro de 2021. **Digestibilidade ileal verdadeira do fósforo do farelo de soja para frangos de corte machos e fêmeas**. Orientador: Arele Arlindo Calderano. Coorientadores: Gabriel Cipriano Rocha, Melissa Izabel Hannas e Horacio Santiago Rostagno.

Para determinar o coeficiente de digestibilidade ileal verdadeiro do P do farelo de soja (FS), um total de 384 frangos (Cobb 500) de 21 dias de idade, 192 machos e 192 fêmeas, foram distribuídos em delineamento inteiramente casualizado em esquema fatorial 3 x 2 (três níveis de P total x sexo), com oito repetições e oito aves por unidade experimental. Os níveis de P total nas dietas experimentais foram 2,8, 3,5 e 4,2 g / kg. Aumentos nos níveis de P total foram alcançados com a inclusão exclusiva de FS. As dietas foram fornecidas dos 21 aos 26 dias de idade, quando foi feita a coleta da digesta ileal das aves. Após as análises químicas, o conteúdo ileal de P digestível das dietas foi plotado contra a concentração total de P em uma análise de regressão linear. A inclinação da reta de regressão forneceu o coeficiente de digestibilidade ileal verdadeiro do P do FS. Não houve interação entre sexo e P total das dietas ($P > 0,05$). Houve aumento linear do conteúdo de P digestível ileal ($P < 0,001$) com o aumento dos níveis de P total nas dietas, de acordo com a equação $Y = 0,419X + 2,350$. Em conclusão, o coeficiente de digestibilidade ileal verdadeiro do P do FS estimado para frangos de corte machos e fêmeas foi de 0,419.

Palavras-chave: Digestibilidade. Fósforo. Frango de corte. Soja como alimento.

SUMÁRIO

TRUE ILEAL PHOSPHORUS DIGESTIBILITY OF SOYBEAN MEAL FOR MALE AND FEMALE BROILER CHICKENS

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Abbreviations: P, phosphorus; SBM, soybean meal; DM, dried matter; CP, crude protein; Ca, calcium; AIA, acid-insoluble ash; DWG, daily weight gain; DFI, daily feed intake.

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1. Introduction

Phosphorus (P) is an important macromineral present in ingredients of plant and animal origin and inorganic phosphates. Compared to other two sources, the P concentration in plant-based ingredients, such as soybean meal (SBM), is low. However, these ingredients are the major constituents of poultry diets and thus provide a significant amount of total dietary P. Approximately two-thirds of the total P present in plant ingredients is bound to phytic acid. However, the poultry's ability to use this P is low due to the lack of endogenous phytase (Selle and Ravindran, 2007).

The nonphytate P of a feed ingredient is calculated by subtracting the analyzed phytate P from the total P content. The term nonphytate P has been used in poultry nutrition as the available P (NRC, 1994). However, the prececal digestibility or true ileal digestibility of P has been suggested as the best method to access the real P availability for poultry (WPSA, 2013). This measure refers to the P of the diet not recovered in the ileum terminal content, not being influenced by postileal microbial activity and by P excretion via the urine (Ravindran et al., 1999; Shastak et al., 2012).

The assessment of true ileal P digestibility in ingredients used in poultry diets is an emerging area of research; however, there are still limited published data available in this area (Mutucumarana and Ravindran, 2016; Ghazaghi et al., 2019; Hanna et al., 2020). In addition, there are gaps in knowledge about the factors that can influence the ileal P digestibility of ingredients, such as the sex of broilers. Previous studies have reported that males retain more phytate P than females at various dietary Ca levels (Edwards et al., 1989). Ziaei et al. (2007) reported a P retention of 0.55 and 0.49 g/bird/d for male and female respectively, suggesting that sex can influence phytate-P degradation.

The true ileal P digestibility for a given ingredient can be tested by regression analysis. This implies that a low-P basal diet is used, and a minimum of two other levels of the P

source are tested. At the highest level of inclusion, the P supply must not exceed the bird's requirement. These conditions allow the determination of the true ileal P digestibility coefficient of the test source from the linear regression slope. This implies that correction for basal endogenous P losses is not necessary (WPSA, 2013).

The objective of this study was to determine the true ileal P digestibility coefficient of SBM for male and female broiler chickens.

2. Materials and methods

2.1. Ethical Matters

The Animal Care and Use Committee of the *Universidade Federal de Viçosa*, Brazil, approved all animal-handling procedures (protocol no. 85/2019). The experiment was conducted according to the experimental protocol for the use of live birds from the Brazilian College of Animal Experimentation.

2.2. SBM

The SBM used in this study was purchased from a local commercial source. A representative sample was collected for analysis of dry matter (DM; method 930.15; AOAC International, 2012), crude protein (CP; method 45; Brazilian Compendium of Animal Nutrition, Sindiracões, 2013), ether extract (method 12; Brazilian Compendium of Animal Nutrition, Sindiracões, 2017), crude fiber (method 18; Brazilian Compendium of Animal Nutrition, Sindiracões, 2017), ash (method 5; Brazilian Compendium of Animal Nutrition, Sindiracões, 2017), calcium (Ca; method 968.08D; AOAC International, 2012), total P (method 968.08D; AOAC International, 2012), phytate P (Near Infra-Red Reflectance Spectroscopy - NIRS), KOH-soluble protein (method 50; Brazilian Compendium of Animal Nutrition, Sindiracões, 2013), urease activity (method

2; Brazilian Compendium of Animal Nutrition, Sindiracões, 2013), and intrinsic phytase activity (ISO 30024:2009; FEFANA; Table 1).

2.3. Broilers and experimental design

Day-old male and female broiler chickens (Cobb 500) were obtained from a commercial hatchery (Rivelli Alimentos SA, Matheus Leme, MG, Brazil). The birds were vaccinated against bursal disease and Marek's disease (Serotype 3, Live Marek's Disease Vector, Merial Inc., Athens, GA). From one day old until the beginning of the experiment, the birds were reared on floor pens equipped with nipple drinkers and a feed dispenser. They had free access to water and were fed ad libitum with a corn/SBM-based standard mash diet formulated to meet their nutritional requirements according to NRC (1994), including for P and Ca levels, with the absence of coccidiostats and antibiotics growth promoters.

At 21 days old, a total of 384 broiler chickens, 192 males (952 ±59 g) and 192 females (892 ± 44 g), were distributed based on their body weight in a 3 x 2 (three total P levels x sex) factorial completely randomized design, with eight replicates per treatment and eight birds per experimental unit. Birds were housed in wire floor cages (500 cm2/bird) in a four-level battery equipped with a trough feeder and a nipple drinker. Birds were exposed to 20 h of light per day, and room temperature was maintained at approximately 22 ◦C during all experiment. Daily weight gain (DWG) and daily feed intake (DFI) were recorded at 26 days old.

Table 1. Composition of SBM, as fed basis

Dry matter (g/kg)	879.5
Crude protein (g/kg)	463.0
Ether extract (g/kg)	14.9
Crude fiber (g/kg)	62.1
Ash (g/kg)	66.1
Calcium (g/kg)	2.90
Total P (g/kg)	6.00
Phytate P (g/kg)	4.06
Nonphytate P[a] (g/kg)	1.94
KOH protein solubility (%)	83.5
Urease activity (Δ pH)	0.13
Intrinsic phytase activity (U/kg)	< 50

[a] Calculated by subtracting the phytate P from the total P.

2.4. Diets

Three semi-purified diets were formulated using the analyzed values of P and Ca in SBM (Table 1). The calculated levels of total P were 2.8, 3.5 and 4.2 g/kg (Table 2). Increases in total P levels of the diets were achieved with the exclusive inclusion of SBM. The total Ca:P ratio in all diets was maintained at 1.35 by the addition of limestone (38.3 % Ca, as fed basis). The addition of SBM and limestone in diets 2 and 3 was carried out to replace starch. All diets contained 10 g/kg celite (acid-insoluble ash - AIA) as an indigestible marker. Growth promoters and coccidiostats were not used in the experimental diets. The diets were offered in mash form. From 21 days of age, the diets were offered *ad libitum* for 5 days to birds.

2.5. Digesta collection and processing

On day 26, six hours after the lights were turned on, all birds were euthanized by cervical dislocation, and the ileal digesta were collected and processed. The collection region of the ileum was between Meckel's diverticulum and 2 cm before the ileo cecal junction. Digesta were flushed out with reverse-osmosis water, pooled within a cage, frozen immediately and subsequently lyophilized. Diet and digesta samples were ground

to pass through a 0.5-mm sieve and stored in airtight plastic containers until chemical analysis.

2.6. Chemical analysis

Representative samples of diets and ileal digesta were analyzed for DM (method 930.15; AOAC International, 2012), Ca (method 968.08D; AOAC International, 2012), total P (method 968.08D; AOAC International, 2012), and AIA (Van Keulen and Young, 1997).

2.7. Calculations

First, ileal P digestibility of SBM was calculated for each diet and replicate according to the following equation:

$$\text{Ileal P digestibility (\%)} = 100 - [100 \times (AIA_{diet} \times P_{digesta}) / (AIA_{digesta} \times P_{diet})]$$

where AIA_{diet} is the acid-insoluble ash concentration in the diet (g/kg DM), $P_{digesta}$ is the P concentration in the digesta (g/kg DM), $AIA_{digesta}$ is the acid-insoluble ash concentration in the digesta (g/kg DM), and P_{diet} is the P concentration in the diet (g/kg DM).

The ileal P digestibility calculated in the first equation was used to calculate the content of ileal digestible P for each the diets, as follows:

$$\text{Ileal digestible P (g/kg DM)} = \text{Ileal P digestibility (\%)} \times P_{diet} / 100$$

Table 2. Ingredients and nutrient composition of experimental diets, as fed basis (g/kg)

Ingredients	Level 1	Level 2	Level 3
Soybean meal	466.7	583.4	700.0
Starch	395.8	277.5	159.3
Sucrose	80.0	80.0	80.0
Soybean oil	30.0	30.0	30.0
Salt	5.0	5.0	5.0
Limestone	6.3	7.9	9.5
DL-Methionine (99%)	3.0	3.0	3.0
Celite	10.0	10.0	10.0
Trace mineral premix[a]	1.0	1.0	1.0
Vitamin premix[b]	1.2	1.2	1.2
Choline chloride (60%)	1.0	1.0	1.0
Calculated composition			
Metabolizable energy (MJ/kg)	12.71	12.06	11.42
Crude protein	216.1	270.1	324.1
Calcium	3.78	4.73	5.67
Total phosphorus	2.80	3.50	4.20
Ca:total phosphorus	1.35	1.35	1.35
Digestible lysine	1.18	1.48	1.78
Digestible methionine	0.55	0.60	0.68
Analyzed values			
Calcium	3.40	4.46	5.40
Total phosphorus	2.80	3.50	4.00
Ca:total phosphorus	1.21	1.27	1.35

[a] Trace mineral premix provided per kg of diet: Mn, 58.36 mg; Zn, 54.21 mg; Fe, 41.68 mg; Cu, 8.31 mg; I, 0.843 mg; Se, 0.250 mg.
[b] Vitamin premix provided per kg of diet: vitamin A, 11,564 UI; vitamin D_3, 2,891 UI; vitamin E, 43.3 UI; vitamin K_3, 2.32 mg; vitamin B1, 3.11 mg; vitamin B12, 0.019 mg; vitamin B6, 4.33 mg; vitamin B5, 15.5 mg; vitamin B3, 47.0 mg; vitamin B9, 1.08 mg; biotin, 0.11 mg.

The content of ileal digestible P (g/kg DM) of the diets was plotted against total P concentration (g/kg DM) in a linear regression analysis. The slope of the regression line represented the true ileal P digestibility coefficient of SBM.

2.8. Statistical analyzes

A 2 × 3 factorial arrangement of treatments was used to investigate the response of male and female broiler chickens to three levels of total P in the diets. Data were analyzed using the GLM procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Cage served

as the experimental unit, and differences were considered significant at an alpha level of 0.05. A linear regression model was used to assess the effects of total P levels on the evaluated parameters.

3. Results and discussion

The calculated and analyzed P concentrations in the diets were similar, except in diet 3, in which the analyzed value was 0.20 g/kg lower than the calculated value (Table 2).

Broilers remained healthy during the five-day experimental period and no mortality and leg problems were recorded. There was an interaction between sex and total P of diets only on DWG of broilers ($P = 0.027$; Table 3). For males and females the DWG increased linearly with increasing levels of total P in diets ($P < 0.001$ and $P = 0.033$, respectively). This effect may be result of the increased nutrient intake. In addition to the increase in P and Ca levels, the level of CP in diets increased by 216.1–324.1 g/kg due to the inclusion of SBM.

There were linear increases in DFI ($P = 0.023$) with increasing levels of total P in the diets. Similarly, Trairatapiwan et al. (2018) found that the body weight gain and feed intake of broilers increased with increases in the inclusion level of SBM in a semipurified diet. The present results also coincide with the findings of Li et al. (2020), who reported that broilers fed a P-deficient diet had the lowest average daily feed intake and average daily gain.

In the present study the analyzed Ca:total P ratio ranged from 1.21 to 1.35 in experimental diets. It has been reported that there are no difference in P digestibility in diets containing Ca:P ratios between 1.2 and 2.0 (Liu et al., 2013).

There were linear increases in the content of ileal P digestible ($P < 0.001$) and a linear decrease in the ileal P digestibility coefficient ($P < 0.001$) with increasing levels of total P in the diets. The equations are presented in Table 4. The reduction in ileal P digestibility

coefficient observed may be related to increasing concentrations of phytate with increasing inclusion levels of SBM (Trairatapiwan et al., 2018). The analyzed phytate P of SMB used in this study was 4.06 g/kg. This represents 67.7 % of the total P of this ingredient. This is in accordance with published data of phytate P in SMB (3.7–4.7 g/kg; Liu et al., 2012; Mutucumarana et al., 2015). In our study, the average ileal P digestibility coefficients to SBM varied from 0.76 in the diet 1 (2.80 g/kg of total P) to 0.66 (4.00 g/kg of total P). These results are consistent with those obtained in several experiments with diets ranging from 3.02 to 4.59 g/kg of total P, and the ileal P digestibility coefficients varied from 0.82 to 0.45, respectively (Rodehutscord et al., 2017).

The true ileal P digestibility coefficient estimated for male and female broiler chickens was 0.419. Considering that the SBM used in this study had 6.00 g/kg of total P, from this coefficient, the content of the true estimated ileal P digestible is 2.51 g/kg. This value is greater than the nonphytate P value of 1.94 g/kg calculated by subtracting the analyzed phytate P from the total P content. These results suggest that part of the phytate P present in the SBM is made available to birds, possibly by intrinsic phytase activity.

The true ileal P digestibility coefficient estimated in the present study was similar to those observed by Trairatapiwan et al. (2018), who estimated a true ileal P digestibility coefficient of 0.423. However, it was lower than the value of 0.523 observed by Mutucumarana et al. (2015). Both studies used the regression method. In addition to the total P concentration in experimental diets, the difference between studies regarding P digestibility maybe related to the P and Ca concentration in the diets provided to the birds in the pre-experimental phase.

The present study showed that the sex of broiler does not influence the true ileal P digestibility coefficient of SBM. In addition, our results reinforce that the determination

of true ileal P digestibility tested by a regression analysis is the better method to access the real P availability of plant ingredients for poultry compared to nonphytate P calculated by subtracting the analyzed phytate P from the total P content. Further studies to determine other factors that may influence the true ileal P digestibility are encouraged.

Table 3. Daily weight gain (DWG) and daily feed intake (DFI) of male and female broilers, ileal P digestibility coefficients (IPDC), and ileal P digestible (IPD)[a].

	Male			Female				*P*-value		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	SEM[b]	Sex	Total P level	Sex x Total P level
DWG (g/bird)	72.3	85.5	86.2	72.3	79.5	76.8	1.05	<0.001	<0.001	0.027
DFI (g/bird)	102.3	110.8	109.8	100.3	106.5	99.4	1.18	0.011	0.023	0.251
IPDC	0.78	0.72	0.65	0.74	0.69	0.67	0.01	0.090	<0.001	0.099
IPD (g/kg DM)	2.40	2.77	2.84	2.28	2.62	2.92	0.04	0.153	<0.001	0.105

[a] Each value represents the mean of eight replicates (eight birds/replicate)
[b] Standard error of the mean.

Table 4. Linear relationship between daily feed intake (DFI), ileal P digestibility coefficient (IPDC) and ileal P digestible (IPD) vs. dietary P content (g/kg DM)

	Regression equation	SE of the slope[a]	SE of the intercept[a]	r^2	True ileal P digestibility coefficient
DFI	$Y = 3.03X + 102.75$	2.21	1.91	0.04	-
IPDC	$Y = -0.079X + 0.764$	0.012	0.010	0.50	-
IPD	$Y = 0.419X + 2.350$	0.044	0.038	0.65	0.419

[a] Standard error of regression.

4. Conclusion

The true ileal P digestibility coefficient in SBM estimated for male and female broiler chickens was 0.419.

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UNIVERSIDADE FEDERAL DE VIÇOSA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE PRODUÇÃO
CEUAP/UFV

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Viçosa, 26 de Jun. de 2019

CERTIFICADO

Certificamos que o projeto intitulado **"Digestibilidade pré-cecal de fósforo do farelo de soja para frangos de corte"**, protocolo **nº 085/2019**, sob a responsabilidade de **Arele Arlindo Calderano** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo chordata, subfilo vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 8 de outubro de 2008, do decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo conselho nacional de controle da experimentação animal (concea), e foi apreciado pela comissão de ética no uso de animais de produção da universidade federal de viçosa (ceuap-ufv) em reunião de **17 de Jun. de 2019.**
Finalidade: **(x)Pesquisa ( )Ensino**
Vigência do Projeto: de **26 de Jun. de 2019** a **30 de dezembro de 2019**
Espécie/linhagem: **Frango de corte (*Gallus gallus domesticus*)** Nº de animais: **384**
Peso: **0,900 kg** Idade: **21 dias** Sexo: **Macho/Fêmea** Origem: **Incubatorio Rivelli Cnpj/CPF: 478.715.616-49 / Endereço: Rua Leão José, 257 Mateus Leme, MG Responsável : Maria Cecília CRMV: 10595**

CERTIFICATE

We certify that the project entitled **"Pre-caecal phosphorus digestibility of soybean meal for broiler chickens"**, protocol **nº 085/2019**, under the responsibility of **Arele Arlindo Calderano** - which involves the production, maintenance and/or use of animals belonging to the phylum chordata, subphylum vertebrata (except man), for scientific research purposes (or education) - is in accordance with the law nº. 11.794, of October 8, 2008, Decree nº. 6899 of July 15, 2009, and the rules issued by the Brazilian National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (CEUAP-UFV) in its meeting on **Jun, 17th, 2019**.
Finality: **(x)Research ( )Education**
Duration of the Project: from **Jun, 26th, 2019** to **Dec. 30th, 2019**.
Species / strain: **Broiler (*Gallus gallus domesticus*)** Nº of animals: **384**
Weight: **0,900 kg** Age: **21 days** Sex: **Male/ Female** Source: **Incubatorio Rivelli Cnpj/CPF: 478.715.616-49 / Endereço: Rua Leão José, 257 Mateus Leme, MG Responsável : Maria Cecília CRMV: 10595**

Luciana Navajas Rennó
Coordenadora da CEUAP/UFV