

# Research Note: Amylase supplementation improves starch and amino acids digestibility of faba bean for broilers

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**ABSTRACT** This study aimed to investigate the effect of amylase on the nutritional value and nitrogen-corrected apparent metabolizable energy ( $AME_N$ ) of faba bean (**FB**) seeds in broiler chicken nutrition. The study was conducted on a total of 84, one-day-old male broiler chickens (Ross 308). The birds were fed a reference corn-soybean meal diet from d 1 to 16, and they were divided into three groups (1st day). The first group (1) was fed the same reference diet as in the first part, whereas 50% of the reference diet was replaced by FB seeds in experimental groups (2 and 3). Group 3 was supplemented with amylase, which contained 0.14 g/kg dry matter (**DM**). At the end of the study period,

excreta and digesta samples were collected from all groups to estimate the nutritional value. Some fraction of starch found in the seeds was part of starch which is resistant to digestion (143.9 g/kg DM). The addition of exogenous amylase to the diet increased the apparent ileal digestibility of DM, CP, and starch in FB seeds. The  $AME_N$  value increased as a result of enzyme addition ( $P < 0.05$ ). The group 3 showed higher apparent ileal digestibility of essential amino acids (Lys, Leu, Phe, His) as well as nonessential amino acids (Tyr, Ala, Pro) in FB seeds. It could be concluded that FB seeds supplemented with exogenous amylase have a greater nutrition value for broiler chickens.

**Key words:** faba bean, amylase, nutritional value, broiler chicken

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## INTRODUCTION

Faba bean (**FB**) is a potential native protein source for poultry. The FB varieties that were formerly cultivated contained a high level of antinutritional factors (**ANF**). Also, faba bean seeds are rich in starch which is the most important energy source in the diet of broiler chickens. Starch present in legumes is of type C, also some fraction of legume starch is not digestible by endogenous enzymes and hence referred to as resistant starch (**RS**; Cai et al., 2014). Factors influencing the digestibility of starch include the ratio of amylase to amylopectin and the diameter of starch granules. Therefore, RS is mainly defined as a factor limiting the potential energy value of nutritional ingredients.

A reduction in the level of RS by extrusion can contribute to improving the digestibility of starch (Hejdysz et al., 2017) and enhancing growth performance-

related factors such as feed intake and feed conversion ratio (Hejdysz et al., 2019a), as the treatment temperature changes the chemical structure of starch. An alternative to this process could be enzyme supplementation in diets. Data on the direct impact of single amylase application in a FB-based diet are scarce. However, supplementation with amylase was shown to improve the value of nitrogen-corrected apparent metabolizable energy ( $AME_N$ , Stefanello et al., 2019) in standard corn-soybean diets. The greater  $AME_N$  value was directly correlated with higher digestibility of nutrients such as ether extract (**EE**), CP, and starch, and also with better growth performance parameters such as body weight gain and feed conversion ratio (Stefanello et al., 2019). On the other hand, Liu et al. (2020) did not observe any effect of amylase on the digestibility of starch in a corn-based diet in their study.

Therefore, we hypothesized that amylase supplementation will improve the nutritional value of FB used for broiler chickens. We aimed to compare the effect of amylase supplementation of FB seeds on the apparent ileal digestibility of nutrients and amino acids,  $AME_N$  value, and excretion of sialic acid in broiler chickens.

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## MATERIALS AND METHODS

All animal procedures were carried out in accordance with the guidelines of the Polish Council of Animal Care (Polish Republic 2015).

### Faba Bean Seeds

Raw FB seeds (*Vicia faba* L.) cv. Amulet were harvested in Poland in 2019 and obtained from plant breeding stations (Strzelce, Poland). FB seeds were ground in a hammer mill (RG11 model, Zuptor, Gostyn, Poland), with a screen size of 2.0 mm. The chemical composition of the faba bean seeds is presented in [Table 1](#).

### Diets, Bird Management, and Sample Collection

The study used a completely randomized experimental design with a control group and 2 experimental groups (without and with addition of amylase). The total of 84, one-day-old male broiler chickens (Ross 308) were included into study. The birds (initial individual

weight:  $40 \pm 2$  g) were obtained from a commercial hatchery (DanHatch Poland, Wolsztyn, Poland). They were randomly divided into 3 groups (1st day) and placed in metabolic cages. Each experimental group consisted of 14 replications (2 birds in each). The birds were maintained under controlled environmental conditions in accordance with the Ross 308 producer recommendations and had unlimited access to feed and freshwater. The composition of the standard corn-soybean meal diet (reference,  $AME_N - 12.85$  MJ/kg; CP - 218 g/kg) was earlier presented in [Hejdysz et al. \(2016\)](#). All diets were formulated with the recommendations of producer. All birds were fed a reference from d 1 to 16 of the experiment. Then, birds in groups 2 and 3 were fed a reference diet containing FB seeds mixed in a 50:50 proportion, and birds in group 1 was still fed reference diet. The enzyme alpha-amylase (RONOZYME Histarch, DSM Nutritional Products, Heerlen, Netherlands) was added to diet of group 3 at a level of 0.14 g/kg as fed. Titanium dioxide was added to diets at 3 g/kg as fed. Collection trays were installed in each metabolic cage. Excreta was collected from d 21 to 22 of the experiment (twice per one day). The ileum was excised following cervical dislocation and digesta was flushed from the terminal ileum (15 cm, adjacent to the ileocecal junction) and pooled per cage. The collected digesta and excreta samples were frozen and then lyophilized.

**Table 1.** Chemical composition, antinutritional factors profile and amino acids profile of faba bean seeds (g/kg as fed).<sup>1</sup>

	Item		
Content (g/kg as fed)	Dry matter	872.30	
	Crude protein	284.00	
	Crude fat	12.50	
	Ash	35.90	
	Starch	365.80	
	ADF	120.50	
	NDF	216.00	
	Resistant starch	143.90	
	Essential amino acids (g/kg as fed)	Arg	27.30
		Cys	2.00
Gly		13.60	
His		8.50	
Ile		12.40	
Leu		22.40	
Lys		15.60	
Met		2.00	
Phe		13.00	
Thr		9.80	
Val		13.90	
Nonessential amino acids (g/kg as fed)		Ala	13.60
	Asp	36.20	
	Glu	46.70	
	Pro	5.90	
	Ser	16.40	
	Tyr	9.60	
Non-starch polysaccharides (g/kg as fed)	Total	140.50	
	Arabinose	20.00	
	Xylose	13.60	
	Mannose	0.80	
	Galactose	4.30	
	Glucose	70.20	
Antinutritional factors (g/kg as fed)	Uronic acids	31.60	
	sRFO	40.20	
	Raffinose (% of RFO)	4.53	
	Stachyose (% of RFO)	21.88	
	Verbascose (% of RFO)	73.59	
	Phytic P	2.80	
	Tannins	0.50	

Abbreviations: ADF, acid detergent fiber; DM, dry matter; NDF, neutral detergent fiber; sRFO, sum of oligosaccharides from raffinose family.

<sup>1</sup>Each value represents mean of 2 replicates.

### Chemical Analysis

The seeds were assessed ( $n = 2$ ) for the content of DM, CP, EE, and crude ash (CA), using standard methods (934.01, 976.05, 920.39, 942.05, and 978.10) ([AOAC International, 2007](#)). The content of DM, CP, and starch was also determined in digesta samples, using the above-mentioned methods ([AOAC International, 2005, 2007](#)). In addition, the content of acid detergent fiber (ADF) and neutral detergent fiber (NDF) was estimated in seeds using standard methods (942.05 and 973.18, respectively) ([AOAC International, 2007](#)). The starch content was determined in FB seeds as well as in digesta samples using method 996.11 ([AOAC International 2005](#)) with an assay kit (Megazyme International, Dublin, Ireland) based on thermostable alpha-amylase and amyloglucosidase. The content of RS in FB seeds was analyzed with an RS assay kit (Megazyme International, Wicklow, Ireland) using method 2002.02 ([AOAC International 2005](#)), by modifying the incubation time as described in [Hejdysz et al. \(2017\)](#). The content of amino acids in FB seeds and digesta samples was determined by using procedure 994.12 ([AOAC International, 2007](#)) in an AAA-400 Automatic Amino Acid Analyser (INGOS s.r.o., Prague, Czech Republic). The amount of oligosaccharides from the raffinose family (RFO), phytates, tannins, and titanium dioxide in FB seeds was determined as described in [Hejdysz et al. \(2016\)](#). Gross energy was determined in FB seeds, diets, and excreta samples using an adiabatic bomb calorimeter (KL 12 Mn, Precyzja-Bit PPHU, Bydgoszcz, Poland)

standardized with benzoic acid. The sialic acid determination method is described in Hejdysz et al. (2019a).

## Calculations

The AID of DM, CP, starch, and amino acids in reference diet (group 1) was calculated based on the ratio of titanium dioxide concentration to the content of nutrients in diet and digesta. The difference method approach was used to calculate apparent ileal digestibility coefficients of various dietary components contained in FB (group 2 and 3), using the proportion of the investigated FB seeds in reference diets. The value of AME<sub>N</sub> of the reference diet (group 1) was calculated and corrected to zero nitrogen balance with 34.4 kJ/g N retained, and then the AME<sub>N</sub> (kcal/kg) of FB seeds (group 2 and 3) was calculated by the difference method. The formulas for the calculation of AME<sub>N</sub> and digestibility coefficients were presented earlier in Hejdysz et al. (2017).

## Statistical Analysis

The obtained data were tested to eliminate any possible outliers and analyzed using statistical software (SAS, 2012). The number of repetitions and the number of birds in the repetition were determined using the G\*Power program (version 3.1.9.6). Pooled standard error of the mean and group mean values were calculated. The significance of differences between the mean values of the analyzed factors was determined by a *t* test at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

The overall mortality was low throughout the experiment (<1%) and was not associated with any of the tested dietary treatments (data not shown).

The chemical composition of the FB seeds (cv. Amulet) used in the study is presented in Table 1. Our previous study indicated that the concentration of nutrients in FB seeds was dependent on the cultivars. One of the most important parameters tested in the study was the concentration of starch, and was determined at 365.8 g/kg as fed; however, analyses indicated that RS (143.9 g/kg as fed) constituted about 39% of total starch. FB seeds have been recognized as a rich source of some essential amino acids, such as arginine, leucine, and lysine (27.3, 22.4, and 15.6 g/kg as fed, respectively), but as deficient in methionine and cysteine. The analyses determined the content of some ANF, which could have a negative impact on animal health. The amount of tannins did not exceed 0.5 g/kg as fed. The total amount of RFO was around 40.0 g/kg as fed, with verbascose being the dominant oligosaccharide (73.59%).

The effects of amylase addition on the apparent ileal digestibility of DM, CP and starch, AME<sub>N</sub> value of FB, and excretion of sialic acid are presented in Table 2. The

**Table 2.** The coefficients of apparent ileal digestibility (AID) of dry matter, crude protein, starch, amino acids (AAs) and AME<sub>N</sub> (kcal/kg as fed) level, and the excretion of total and free sialic acid ( $\mu\text{mol/g TiO}_2$ ) from broiler chickens fed faba bean seeds without (-) and with (+) enzyme supplementation.<sup>1</sup>

Item	Amylase		SEM	<i>P</i>
	-	+		
Dry matter	0.639	0.685	0.0124	0.012
Crude protein	0.903	0.940	0.0102	0.017
Starch	0.765	0.795	0.0104	0.045
Essential AAs				
Arg	0.927	0.935	0.0077	0.327
Gly	0.857	0.881	0.0094	0.070
His	0.889	0.918	0.0051	0.021
Ile	0.908	0.923	0.0892	0.239
Leu	0.916	0.945	0.0074	0.021
Lys	0.925	0.958	0.0074	0.003
Phe	0.902	0.936	0.0094	0.017
Thr	0.885	0.880	0.0147	0.833
Val	0.918	0.934	0.0099	0.251
Nonessential AAs				
Ala	0.910	0.942	0.0095	0.027
Asp	0.908	0.917	0.0748	0.417
Glu	0.945	0.961	0.0075	0.158
Pro	0.881	0.921	0.0119	0.019
Ser	0.904	0.927	0.0134	0.235
Tyr	0.905	0.937	0.0092	0.020
AME <sub>N</sub> (kcal/kg as fed)	2300	2420	32.6424	0.014
Excretion of sialic acid:				
Free	410.0	425.0	16.72	0.537
Total	479.0	498.0	17.47	0.445

Abbreviations: AME<sub>N</sub>, nitrogen corrected apparent metabolizable energy; SEM, pooled standard error of mean.

<sup>1</sup>Each value represents mean of 14 replicates.

addition of amylase increased digestibility coefficients of DM by about 7.2%, of CP by about 4.1% and of starch by about 3.9%. Chickens have a relatively short gastrointestinal tract, which limits the time of interaction of digesta with their own enzymes. Thus, enzyme supplementation can cause an increase in the amount of enzyme, allowing the chickens to use starch more efficiently. Also, Gracia et al. (2003) showed an improvement digestibility coefficient of starch and AME<sub>N</sub> value with amylase-supplemented corn–soybean meal diets. To our knowledge, an analysis of the impact of amylase (used as a single enzyme) on the broiler chicken diet based on FB has not been reported so far. Native starch in FB seeds contains a high level (>40%) of amylase and is classified as type C (Cai et al., 2014). Compared to other types, type C starch is more difficult to digest. In the current study, increase in AID coefficients of DM and CP was observed. Furthermore, Woyengo et al. (2019) observed an improvement digestibility coefficient of crude protein associated with the hydrolysis of starch and an increase in the availability of phosphorus-bound protein matrix. The addition of enzyme in FB contributed to a significant increase in the digestibility coefficients of amino acids such as lysine, leucine, phenylalanine, histidine, tyrosine, alanine, and proline ( $P < 0.05$ ). The highest digestibility coefficient was estimated for proline (about 4.54%), whereas the lowest for leucine (about 3.2%). Starch and protein can form mutual complexes (Wang et al., 2020), however, amylase may disrupt these complexes, breaking the

glycosidic bonds, and therefore increase the access of enzymes to proteins and amino acids. It has been reported earlier that a high level of RS is responsible for lower nitrogen retention (Hejdysz et al., 2017; Liu et al., 2020). It can be expected that RS forms complexes with protein, thus decreasing its access to enzymes. Perhaps, the addition of amylase could contribute to preventing the formation of RS–protein complexes. Also, the reduction in the use of amino acids for the synthesis of pancreatic amylase (Garcia et al., 2003) could lead to a reduction in AA losses, which was observed here as a more favorable digestibility of nonessential AAs. However, this finding should be confirmed in future studies.

Amylase addition caused a significant increase in the  $AME_N$  value ( $P < 0.05$ ; +5.2%). This is directly related to the improvement of the digestibility coefficient of starch as well as CP. Stefanello et al. (2019) also found an improvement in the  $AME_N$  value. The greater  $AME_N$  value and higher digestibility coefficient of starch could be perhaps explained by the reduction of RS after enzyme addition.

No significant effect of supplementation on the excretion of free and total sialic acid was observed. The amount of free and total sialic acid excreted was not different in both treatment groups. Earlier studies have shown a reduction in the secretion of sialic acid by broiler chickens fed with extruded FB seeds (Hejdysz et al., 2019a) as a result of lower RS concentration.

In conclusion, this study confirmed the positive effect of amylase on the nutritional value of FB seeds in the broiler chicken diet. Amylase increased the digestibility coefficients of nutrients in particularly starch and some AA, as well as the  $AME_N$  value. Based on the obtained results, it can be suggested that FB seeds supplemented with amylase can be a valuable source of protein and energy for broiler chickens.

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## DISCLOSURES

The authors declare that there are no conflict of interests

## REFERENCES

- AOAC International 2005. 2007. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists International, Gaithersburg, MD.
- Cai, J., C. Cai, J. Man, W. Zhou, and C. Wei. 2014. Structural and functional properties of C-type starches. *Carbohydr. Polym.* 101:289–300.
- Gracia, M. I., M. J. Aranibar, R. Lazaro, P. Medel, and G. G. Mateos. 2003. Alpha-amylase supplementation of broiler diets based on corn. *Poult. Sci.* 82:436–442.
- Hejdysz, M., S. A. Kaczmarek, M. Adamski, and A. Rutkowski. 2017. Influence of graded inclusion of raw and extruded pea (*Pisum sativum* L.) meal on the performance and nutrient digestibility of broiler chickens. *Anim. Feed Sci. Technol.* 230:114–125.
- Hejdysz, M., S. A. Kaczmarek, M. Kubiś, M. Adamski, K. Perz, and A. Rutkowski. 2019a. The effect of faba bean extrusion on the growth performance, nutrient utilization, metabolizable energy, excretion of sialic acids and meat quality of broiler chickens. *Animal* 13:1583–1590.
- Hejdysz, M., S. A. Kaczmarek, and A. Rutkowski. 2016. Effect of extrusion on the nutritional value of peas for broiler chickens. *Arch. Anim. Nutr.* 70:364–377.
- Liu, H., J. O. Sorbara, A. J. Cowieson, R. L. Millan, S. K. Wang, J. L. Wu, and A. M. Kluehner. 2020. Exogenous  $\alpha$ -amylase supplementation reduces the variability of ileal digestible energy in broiler chickens fed complete diets with maize batches of variable protein solubility. *Anim. Feed Sci. Technol.* 268:114610.
- Stefanello, C., S. L. Vieira, P. Soster, B. D. Santos, Y. K. Dalmore, A. Favero, and A. J. Cowieson. 2019. Utilization of corn-based diets supplemented with an exogenous  $\alpha$ -amylase for broilers. *Poult. Sci.* 98:5862–5869.
- Wang, S., C. Chao, J. Cai, B. Niu, L. Copeland, and S. Wang. 2020. Starch–lipid and starch–lipid–protein complexes: a comprehensive review. *Compr. Rev. Food Sci. Food. Saf.* 19:1056–1079.
- Woyengo, T. A., K. Jerez Bogota, S. L. Noll, and J. Wilson. 2019. Enhancing nutrient utilization of broiler chickens through supplemental enzymes. *Poult. Sci.* 98:1302–1309.