

Appraising the dose sensitivity of 21d-broiler chicks to a mono-component protease in high trypsin inhibitor soybean-based diet

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Abstract

Exogenous proteases are promoted for their ability to counteract the negative effects of antinutritional elements such as trypsin inhibitors in broiler chickens. This is because these anti-nutritional substances are proteinaceous, making them candidates for hydrolysis by proteases. However, there still is insufficient knowledge on the sensitivity of this phenomena to protease dosing, hence this study was designed to evaluate the dose sensitivity of broiler starter chickens fed a high trypsin inhibitor soybean-based diet supplemented with a mono-component protease. Two hundred and sixty-four one-day-old chicks were divided into three treatments, each consisting of eight replicates and 11 chicks per replicate. The control group (C) were offered a corn-soybean-based diet contained 8.47 mg/g of trypsin inhibitor, while the other two groups were offered the control diet supplemented with 250 g/tonne (C+250) and 500 g/tonne (C+500) of a mono-component protease, Cibenza DP100, for 21 days. Blood was sampled for serum albumin, uric acid, creatinine, and total protein, and pancreas, liver, and mid-sections of the jejunum, and ileum were harvested for examination of organ and gut morphology. The findings demonstrated that feed conversion ratio improved with protease dosage, with the C+500 group demonstrating the highest efficiency (1.48 ± 0.22). Serum albumin, total protein, and uric acid levels also increased significantly ($p < 0.05$) with birds fed diet C+500 having the highest values. In contrast, serum creatinine was significantly ($p < 0.05$) higher in the control group. Protease supplementation also increased ($p < 0.05$) villi height and crypt depth in both ileum and jejunum. The relative weight of the liver increased ($p < 0.05$) while that of pancreas decreased ($p < 0.05$) with supplemental protease. In conclusion, these findings suggest that supplementation of a high trypsin inhibitor soybean-based diet with a mono-component protease at 500g/tonne complete diet could be used to achieve better performance in 21-d old chickens.

Keywords: Serine protease, Meat-type chicken, In-feed enzymes, Enzyme superdosing

Running title: Broiler chicks' response to protease dosing



Évaluation de la sensibilité à la dose de poussins de chair 21d à une protéase monocomposant dans un régime à base de soja à inhibiteur élevé de trypsine

Résumé

Les protéases exogènes sont promues pour leur capacité à contrer les effets négatifs des éléments antinutritionnels tels que les inhibiteurs de la trypsine chez les poulets à griller. En effet, ces substances anti-nutritionnelles sont protéiques, ce qui en fait des candidats à l'hydrolyse par les protéases. Cependant, les connaissances sur la sensibilité de ce phénomène au dosage de la protéase sont encore insuffisantes. Cette étude a donc été conçue pour évaluer la sensibilité à la dose de poulets de chair nourris avec un régime à base de soja à haute teneur en inhibiteur de trypsine, complété par une protéase à un seul composant. Deux cent soixante-quatre poussins d'un jour ont été divisés en trois traitements, chacun consistant en huit répétitions et 11 poussins par répétition. Le groupe témoin (C) s'est vu proposer un régime à base de maïs-soja contenant 8,47 mg/g d'inhibiteur de trypsine, tandis que les deux autres groupes se sont vu proposer le régime témoin supplémenté en 250 g/tonne (C+250) et 500 g/tonne (C+500) d'une protéase mono-composant, Cibenza DP100, pendant 21 jours. Le sang a été prélevé pour l'albumine sérique, l'acide urique, la créatinine et les protéines totales, et le pancréas, le foie et les parties médianes du jéjunum et de l'iléon ont été prélevés pour l'examen de la morphologie des organes et

de l'intestin. Les résultats ont démontré que le taux de conversion alimentaire s'améliorait avec le dosage de la protéase, le groupe C+500 démontrant l'efficacité la plus élevée ($1,48 \pm 0,22$). Les taux d'albumine sérique, de protéines totales et d'acide urique ont également augmenté de manière significative ($p < 0,05$), les oiseaux nourris au régime C+500 ayant les valeurs les plus élevées. En revanche, la créatinine sérique était significativement ($p < 0,05$) plus élevée dans le groupe témoin. La supplémentation en protéase a également augmenté ($p < 0,05$) la hauteur des villosités et la profondeur des cryptes dans l'iléon et le jéjunum. Le poids relatif du foie a augmenté ($p < 0,05$) tandis que celui du pancréas a diminué ($p < 0,05$) avec la protéase supplémentaire. En conclusion, ces résultats suggèrent que la supplémentation d'un régime à base de soja à haute teneur en inhibiteur de trypsine avec une protéase monocomposant à 500 g/tonne de régime complet pourrait être utilisée pour obtenir de meilleures performances chez les poulets âgés de 21 jours.

Mots-clés : Sérine protéase, Poulet de type viande, Enzymes alimentaires, Surdosage enzymatique

Introduction

Soybean meal remains the most used plant protein feedstuff in chicken diets due to its well-balanced amino acids and high digestibility (Doskovič *et al.*, 2013). Soybean meal fulfills approximately 80% of the protein and amino acid requirements for all types of poultry and swine at every stage of production (NRC, 1994). Soybeans contain various bioactive substances, including enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds. These substances play metabolic roles in humans or animals that frequently consume soy-based foods. The effects of these substances can be considered positive, negative, or both (Champ, 2002). Some of these substances, such as trypsin inhibitors, have been classified as anti-nutritional factors due to their impact on diet quality (Boudry *et al.*, 2004; Kaczmarek *et al.*, 2014; Erdaw *et al.*, 2017). Soybean seeds require pre-heat treatment to inactivate trypsin inhibitors (Wedekind *et al.*, 2020; Jiang *et al.*, 2021; Takacs *et al.*, 2022), however, variability in processing conditions among factories has led to soybean products with varying nutritional characteristics and trypsin inhibitor concentrations (Karr-Lilienthal *et al.*, 2005). According to Clarke and Wiseman (2005, 2007), trypsin inhibitory activity in soybean products should not exceed 4.0mg/g, as trypsin inhibitor activity above this threshold is correlated with decreased digestibility, rapid feed passage, pancreas hypertrophy, and higher endogenous amino acid losses (Perilla *et al.*, 1997; Boudry *et al.*, 2004; Pacheco *et al.*, 2014). The physiological demands on starter broilers to reach target weight may also make them

particularly sensitive to variability in trypsin inhibitor activity (Clarke and Wiseman, 2005).

The inclusion of exogenous feed enzymes in animal diets has been considered an effective strategy to improve protein efficiency and reduce feed costs in animal production (Adeola and Cowieson, 2011; Erdaw *et al.*, 2017a; Kim *et al.*, 2021). Microbial protease potentially acts by outcompeting trypsin inhibitors (TI) for active sites, thereby improving overall protein and amino acid utilization, or by hydrolyzing proteinaceous anti-nutrients and antigenic proteins (Zuo *et al.*, 2015; Walk *et al.*, 2019; Mohammadigheisar *et al.*, 2021).

While previous studies have investigated the dose effect of enzyme cocktails composed of xylanase, amylase, and protease on the performance and nutrient utilization of broiler starters fed a maize-soy-based diet, limited information is available on the dose effect of mono-component exogenous protease in a high trypsin inhibitor soybean-based diet. Therefore, this study hypothesizes that dosage of a mono-component protease supplementation in a high trypsin inhibitor (TI = 8.47 mg/g) soybean-based diet will impact feed conversion ratio, intestinal histology and indices of protein metabolism in 21d broiler chickens.

Materials and methods

The protocol of the experiment reported herein was reviewed and approved in the Department of Animal Science, University of Ibadan.

Experimental diets, birds, and housing

A high trypsin inhibitor corn-soy-based diet (TI = 8.47 mg/g) was created to fulfil or surpass the breeders' nutritional requirements and served as the control (C). To this diet, a mono-component

protease with protease activity of 600,000units/gram (Cibenza® DP100, Novus International) was added at 250 g/tonne (C+250), and 500 g/tonne (C+500) of complete feed. A total of 264 one-day-old broiler chicks were obtained from a commercial hatchery, individually tagged, weighed, and randomly allotted to 24 groups of 11 chicks each, by weight equalization (Kim and Lindemann, 2007). Each group of chicks was offered one of the three experimental diets. The chicks were raised on deep litter in an open-sided, concrete-floored, naturally illuminated (14L,10D) and ventilated broiler house, with wood shavings as bedding material at a density of 5.75 kg/m². Each pen had dimensions of 0.8 m × 0.8 m and was equipped with a floor drinker and plastic feeder. The temperature within the broiler house was maintained at 32°C up to 7d, while ambient temperature (24-29°C) was maintained for the rest of the study period. Water and feed were offered *ad-libitum*, and the required veterinary vaccination regime against Marek's disease, Infectious bursal disease, and Newcastle disease were observed.

Sampling, data collection, and analyses

On 14d of the study, two chickens per replicate were transferred into metabolic cages for excreta collection for three days. The excreta voided was pooled per cage, and aliquots were collected in triplicates, extracted in distilled water (w/w) and centrifuged at 15,000 rpm. The extract was assayed for uric acid using the uricase PAP test (RANDOX Laboratories Ltd, Crumlin, United Kingdom).

On 21d of the study, 2 mL blood was sampled from two chickens per replicate into plain vials, left to sit for 30min then centrifuged at 15,000 rpm for 15 minutes to extract the serum. Serum was then assayed for total protein, albumin, creatinine, and uric acid using appropriate assay kits (RANDOX Laboratories Ltd, Crumlin, United Kingdom). Thereafter, the chickens were sacrificed by severing the jugular vein with a sharp knife, pancreas and liver were excised and weighed, and *pectoralis major* and *perinous longus* were harvested and assayed for crude protein and crude fat (AOAC, 1990, 2006). Mid-jejunal and mid-ileal segments of the gastrointestinal tract were also harvested,

Table 1. Gross composition and calculated nutrients of experimental broiler starter diet

Ingredients (g/100g)	High trypsin inhibitor soybean-based diet (Control, C)	C + 250 diet	C + 500 diet
Soybean meal	17.50	17.50	17.50
Full-fat soya	26.00	26.00	26.00
Corn	45.00	45.00	45.00
Corn bran	7.28	7.28	7.28
Limestone	0.90	0.90	0.90
Dicalcium phosphate	2.00	2.00	2.00
Choline chloride	0.10	0.10	0.10
Sodium bicarbonate	0.30	0.30	0.30
Vit-mineral premix*	0.25	0.25	0.25
Lysine- HCl	0.30	0.30	0.30
L-Methionine	0.22	0.22	0.22
Table salt	0.15	0.15	0.15
Protease	-	+	++
Calculated Analysis ¹			
Metabolisable energy (kcal/kg)	3024	3024	3024
Crude protein (%)	23.00	23.00	23.00
Ether extract (%)	6.74	6.74	6.74
Crude Fibre (%)	4.28	4.28	4.28
Calcium (%)	1.08	1.08	1.08
Phosphorus (%)	1.73	1.73	1.73
Methionine (%)	0.54	0.54	0.54
Lysine (%)	1.42	1.42	1.42

*Composition of vitamin mineral premix per kg/diet: vitamin A, 10,000 IU; vitamin D₃, 2,000 IU; vitamin E, 32 mg; vitamin K₃, 1.6 mg; vitamin B₁, 2.5 mg; vitamin B₂, 4.4 mg; niacin, 44 mg; calcium pantothenate, 9.2 mg; vitamin B₆, 4 mg; vitamin B₁₂, 0.02mg; choline chloride, 400 mg; folic acid, 0.8 mg; biotin, 0.064 mg; manganese, 96 mg; iron, 80 mg; zinc, 64 mg; copper, 6.8 mg; iodine, 1.2 mg; cobalt, 0.24 mg; selenium, 0.096 mg; antioxidant, 96 mg.

C+250 = High trypsin inhibitor soybean-based diet + 250g Cibenza® DP100/tonne

C+500= High trypsin inhibitor soybean-based diet + 500g Cibenza® DP100/tonne;

Cibenza® DP100 is a mono-component protease produced by Novus International.

¹Calculated analysis based on nutritional values of feed ingredients (NRC, 1994; Feedipedia).

flushed with distilled water, and stored in 10% formalin for subsequent histological assessments. Sections of the jejunum and ileum sampled were stained with hematoxylin and eosin using standard paraffin embedding, image processing and analysis procedures ((Parlee *et al.*, 2014; Optimus software version 6.5; Media Cybergenetics, North Reading, MA).

A representative sample of 200g of litter was also collected from each pen into freezer bags on 21d of the study and stored at -20°C. Litter ammonia concentration was analysed using the micro-diffusion method (Hernandes and Cazetta, 2001). Feed conversion ratio (FCR) was estimated as the ratio of feed consumed per chicken to their body weight gain over 21 days.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) with diets as the treatment factor, and significant means were separated using the Duncan test, with significance based on a 5% probability level (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

Results and Discussion

The effect of protease dosage on feed conversion, organ weights and carcass composition in broiler starter chickens fed a high trypsin inhibitor soybean-based diet are shown in Table 2.

Table 2. Effect of exogenous protease dosage in a high trypsin inhibitor soybean-based diet on feed conversion, organ weights and carcass composition in broiler starter chickens

Variables	High trypsin inhibitor soybean-based diet (Control, C)	C + 250 diet	C + 500 diet	SEM
Feed conversion ratio	1.87 ± 0.09 ^c	1.68 ± 0.11 ^b	1.48 ± 0.22 ^a	0.04
Liver weight (g)	4.05 ± 0.14 ^b	6.14 ± 0.21 ^a	7.23 ± 0.26 ^a	0.39
Pancreas weight (g)	1.78 ± 0.09 ^a	0.94 ± 0.08 ^b	0.82 ± 0.05 ^b	0.10
Carcass composition: Crude protein (%)				
<i>pectoralis major</i>	21.68 ± 1.93	22.90 ± 1.76	22.51 ± 1.62	0.54
<i>peroneus longus</i>	22.41 ± 1.63	20.93 ± 0.95	20.00 ± 0.76	0.49
Carcass composition: Crude fat (%)				
<i>pectoralis major</i>	0.38 ± 0.37	0.17 ± 0.12	0.14 ± 0.12	0.08
<i>peroneus longus</i>	0.68 ± 0.35	0.52 ± 0.26	0.22 ± 0.27	0.11

C+250 = High trypsin inhibitor soybean-based diet + 250g Cibenza® DP100/tonne

C+500 = High trypsin inhibitor soybean-based diet + 500g Cibenza® DP100/tonne;

Cibenza® DP100 is a mono-component protease produced by Novus International.

^{a, b, c} Mean values within a row with no common superscripts differ significantly (P < 0.05).

SEM- Standard error of mean

Protease dosage significantly improved ($p < 0.05$) feed conversion ratio in a high trypsin soybean-based diet by 10.16% in the C+250 treatment and by 20.86% in the C+500 treatment. These results agree with reports of Cowieson and Roos (2016); Erdaw *et al.* (2017a, 2017b); Saleh *et al.*, 2018; Amiri *et al.* (2021); Mohammadigheisar *et al.* (2021) and Tajudeen *et al.* (2022) who reported increased zootechnical performance when proteases were included in high trypsin inhibitor diets. Dietary proteins, antinutritional factors such as trypsin inhibitors and lectins, and antigenic proteins such as glycinin and β -conglycinin are substrates for exogenous proteases, hence the ability of exogenous proteases to mitigate detrimental effects of these antinutritional factors in poultry (Cowieson and Roos, 2016), and enhance protein/true nitrogen digestibility and utilization for growth (Bertechini *et al.*, 2009; Ghazi *et al.*, 2003; Saleh *et al.*, 2018, 2020; Sorbara, 2009). Exogenous proteases may also improve animal performance by decreasing digesta viscosity and improving enzyme-digesta interactions (Erdaw *et al.*, 2017a). Also, exogenous proteases generally impact zootechnical attributes positively in the starter phase of growth (Wang *et al.*, 2008) and could be attributed to severe “rapid feed passage syndrome” induced by trypsin inhibitors in later phases of growth (Ruiz and De Belalcazar, 2005). Sensitivity of the pancreas and liver of chickens to protease dosing was also recorded in the current study with a steady decline ($p < 0.05$) in pancreas weight and increase ($p < 0.05$) in liver weight. Pancreas weight is highly correlated to dietary trypsin inhibitor levels, and increases in pancreas weight are indicative of pancreatic hypertrophy or hyperplasia- a result of compensatory enzyme secretion (especially trypsin and chymotrypsin) by the pancreas to offset the limited concentration and function of trypsin after trypsin inhibitors complex irreversibly with trypsin in the small intestine (Cabrera-Orozco *et al.*, 2013; Pacheco *et al.*, 2014; Aderibigbe *et al.*, 2020). Relative liver weight also correlates well with exogenous protease supplementation and is considered key in growth and development studies especially when protein sources with high trypsin inhibitor concentrations are fed. This is considered a consequence of increased

influx of absorbed nutrients leading to increased nutrient synthesis (Ndazigaruye *et al.*, 2019). Findings in this study are consistent with reports of Rehman *et al.*, (2017) and Erdaw *et al.*, (2018) who recorded higher liver weights in birds fed diets supplemented with microbial proteases. There were however no effects of protease supplementation and dosing ($p>0.05$) on carcass protein and fat composition determined in the breast (*pectoralis major*) and thigh (*peroneus longus*) muscles in this study. Studies have reported significant reduction in carcass protein and fat of 21d broiler chickens fed a high trypsin inhibitor diet of raw soybeans (70 TIU/g DM) compared to low trypsin diets of processed (autoclaved and roasted) soybeans in broiler chickens at 21 days of age (Chohan *et al.*, 1993) hence we opine that the trypsin inhibitor levels in the current study may not have been sufficient to elicit a similar effect.

Serum uric acid, total protein, albumin, and creatinine are indices of protein utilization hence were used to monitor the effects of trypsin inhibitors and the possible mitigating effect of a mono-component protease in different doses. The effect of exogenous protease dosage in a high trypsin inhibitor soybean-based diet on serum, excreta and litter indices are presented in Table 3.

Table 3. Effect of exogenous protease dosage in a high trypsin inhibitor soybean-based diet on serum, excreta, and litter indices of protein metabolism

Variables	High trypsin inhibitor soybean-based diet (Control, C)	C + 250 diet	C + 500 diet	SEM
Serum Uric acid (mg/dL)	9.88 ± 0.31 ^c	10.34 ± 0.16 ^b	11.01 ± 0.47 ^a	0.12
Albumin (g/dL)	1.55 ± 0.17 ^b	1.86 ± 0.07 ^a	1.92 ± 0.05 ^a	0.04
Total protein (g/dL)	2.67 ± 0.79 ^c	3.50 ± 0.60 ^b	4.25 ± 0.41 ^a	0.18
Creatinine (mg/dL)	0.28 ± 0.01 ^a	0.24 ± 0.03 ^b	0.20 ± 0.01 ^c	0.01
Excreta uric acid (mg/g)	130.00 ± 2.60	252.23 ± 43.39 ^b	352.29 ± 9.58 ^a	15.80
Litter Ammonia (ppm)	54.42 ± 5.80 ^b	74.53 ± 16.39 ^a	54.42 ± 9.30 ^b	1.31

^{a, b, c} Mean values within a row with no common superscripts differ significantly ($P < 0.05$).

C+250 = High trypsin inhibitor soybean-based diet + 250g Cibenza® DP100/tonne

C+500 = High trypsin inhibitor soybean-based diet + 500g Cibenza® DP100/tonne;

Cibenza® DP100 is a mono-component protease produced by Novus International.

Significant ($p<0.05$) increases in serum and excreta uric acid concentrations as well as total protein and albumin were observed in the C+250 and C+500 treatments, while serum creatinine decreased ($p<0.05$) with the treatments. Since serum total protein and albumin are considered markers of protein reserves (Hanauska-Brown *et al.*, 2003) and sensitive to protein availability and scarcities, the rise in serum total protein and albumin in response to protease dosage in the current study emphasizes the ability of the mono-component protease to override the trypsin inhibitors' inhibitory effect and increase proteins available from the diet (Allouche *et al.*, 2015; Saleh *et al.*, 2020). It has also been shown that the concentration of plasma albumin relates to improved immunity status in birds (Ding *et al.*, 2016; Saleh 2017; Ndazigaruye *et al.*, 2019). Conversely, serum creatinine is a waste product made by the non-enzymatic conversion of muscle creatine and creatine phosphate considered an indicator of kidney functions. Although a lower concentration of creatinine is observed in the present investigation compared to similar studies (Omar *et al.*, 2021; Elbaz *et al.*, 2023), this may be due to the smaller muscle mass of the chickens associated with their age (Thongprayoon *et al.*, 2016). The concentration of creatinine to protease supplementation in the birds agrees with reports of Dongare *et al.*, 2013. Higher serum and excreta uric acid with higher protease dosage may be due to a higher availability and efficiency of amino acids utilization as uric acid is a product of deamination in poultry species (Donsbough *et al.*, 2010). However, serum uric acid increased with dosage supplementation of exogenous protease (Ndazigaruye *et al.*, 2019). Litter ammonia was significantly different among the treatments but not evidently influenced by protease dosage. In a similar study, litter ammonia at 35d increased ($p<0.05$) by protease supplementation but not ($p>0.05$) by trypsin inhibitor levels of the diet (Erdaw *et al.*, 2017a). The effect of exogenous protease dosage in a high trypsin inhibitor soybean-based diet on histology of mid-jejunum and mid-ileum are presented in Table 4.

Table 4. Effect of exogenous protease dosage in a high trypsin inhibitor soybean-based diet on the histology of the jejunum and ileum of 21d old chicken

Variables (μm)	High trypsin inhibitor soybean-based diet (Control, C)	C + 250 diet	C + 500 diet	SEM
Mid jejunum crypt depth	493.14 \pm 75.08 ^b	575.86 \pm 86.81 ^{ab}	647.63 \pm 166.70 ^a	26.32
villi height	1501.23 \pm 217.09 ^c	1678.39 \pm 111.52 ^b	1995.72 \pm 47.89 ^a	51.03
Mid ileum crypt depth	490.12 \pm 100.00 ^b	519.75 \pm 60.87 ^b	1152.75 \pm 58.78 ^a	65.41
villi height	1234.41 \pm 40.87 ^b	1334.25 \pm 71.07 ^a	1340.03 \pm 55.67 ^a	15.06

^{a, b, c} Mean values within a row with no common superscripts differ significantly ($P < 0.05$).

C+250 = High trypsin inhibitor soybean-based diet + 250g Cibenza® DP100/tonne

C+500= High trypsin inhibitor soybean-based diet + 500g Cibenza® DP100/tonne

Cibenza® DP100 is a mono-component protease produced by Novus International

Inclusion of a protease in the high trypsin inhibitor diet increased ($p < 0.05$) villus height and crypt depth in both jejunal and ileal tissues of the birds in this study, with a similar trend reported in earlier studies (Kamel *et al.*, 2015; Aderibigbe *et al.*, 2020;). Villus height and crypt depth are indices closely associated with nutrient absorption (Choct, 2006) and gut health, with less-than-optimal digestibility of protein feedstuffs that make up the diet adversely impacting intestinal development and function (Gilbert *et al.*, 2008; Wijten *et al.*, 2010). These results indicate that more energy is made available to improve intestinal morphology in the supplemented treatments (Ding *et al.*, 2016).

Conclusion

Supplementation of a high trypsin inhibitor soybean-based diet with a mono-component protease alleviated the negative impact of trypsin inhibitors on feed conversion, protein utilization indices and gastrointestinal histology in 21d old broiler chicken. Dosing of the mono-component protease at 500g/tonne complete diet induced similar or better effects on indices studied compared to dosing at 250g/tonne complete diet.

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References

- Adeola, O. and Cowieson, A.J. 2011.** Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *Journal of Animal Science*, 89: 3189–3218. <https://doi.org/10.2527/jas.2010-3715>
- Aderibigbe, A., Cowieson, A.J., Sorbara, J.O., Pappenberger, G. and Adeola, O. 2020.** Growth performance and amino acid digestibility responses of broiler chickens fed diets containing purified soybean trypsin inhibitor and supplemented with a monocomponent protease. *Poultry Science*, 99(10): 5007–5017. <https://doi.org/10.1016/j.psj.2020.06.051>
- Allouche, L., Madani, T., Hamouda, Z.A., Boucherit, M.R., Taleb, H., Samah, O., Rahmani, K. and Touabti, A. 2015.** Effect of addition of exogenous enzymes in hypocaloric diet in broiler chicken on performance, biochemical parameters and meat characteristics. *Biotechnology in Animal Husbandry*, 31(4): 551–565. <https://doi.org/10.2298/BAH1504551A>
- Almuhanna, E., Ahmed, A and Al-Yousif, Y. 2011.** Effect of air contaminants on poultry immunological and production performance. *International Journal of Poultry Science*, 10(6): 461–470.
- Amiri, M.Y.A., Jafari, M.A. and Irani, M. 2021.** Growth performance, internal organ traits, intestinal morphology, and microbial population of broiler chickens fed quinoa seed-based diets with phytase or protease supplements and their combination. *Tropical Animal Health and Production*, 53(6): 1–8. <https://doi.org/10.1007/S11250-021-02980-0/METRICS>
- AOAC. 1990.** *Protein (Crude) Determination in Animal Feed: Copper Catalyst Kjeldahl Method 984.13*. 15th edition. Gaithersburg, Official Methods of Analysis of AOAC International.
- AOAC. 2006.** *Official Methods of Analysis. 18th*

- ed. Association of Analytical Chemists. Arlington, VA.*
- Barletta, A. 2010.** Introduction: Current market and expected developments. *Enzymes in Farm Animal Nutrition*, 2: 1–11. <https://doi.org/10.1079/9781845936747.0001>
- Bertechini, A., Carvalho, J.C., Mesquita, F., Castro, S., Meneghetti, C. and Sorbara, J.O. 2009.** Use of a protease to enhance the utilization of soybean meal amino acids by broilers. *Poultry Science*, 88(Suppl. 1): 69.
- Boudry, G., Péron, V., Le Huërou-Luron, I., Lallès, J.P. and Sève, B. 2004.** Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. *The Journal of nutrition*, 134(9): 2256–2262. <https://doi.org/10.1093/JN/134.9.2256>
- Cabrera-Orozco, A., Jiménez-Martínez, C. and Dávila-Ortiz, G. 2013.** Soybean: Non-Nutritional Factors and Their Biological Functionality. *Soybean - Bio-Active Compounds*. <https://doi.org/10.5772/52598>
- Champ, M.M.J. 2002.** Non-nutrient bioactive substances of pulses. *British Journal of Nutrition*, 88(S3): 307–319. <https://doi.org/10.1079/BJN2002721>
- Choct, M. 2006.** Reviews Enzymes for the feed industry: past, present and future. *Animal Feed Science and Technology*, 121: 125–138. <https://doi.org/10.1016/j.anifeedsci.2005.02.012>
- Clarke, E. and Wiseman, J. 2005.** Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. *Animal Feed Science and Technology*, 121: 125–138. <https://doi.org/10.1016/j.anifeedsci.2005.02.012>
- Cowieson, A.J. and Ravindran, V. 2008.** Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. *British poultry science*, 49(1): 37–44. <https://doi.org/10.1080/00071660701812989>
- Cowieson, A.J. and Roos, F.F. 2016.** Toward optimal value creation through the application of exogenous mono-component protease in the diets of non-ruminants. *Animal Feed Science and Technology*, 221: 331–340. <https://doi.org/10.1016/J.ANIFEEDSCI.2016.04.015>
- Ding, X.M., Li, D.D., Li, Z.R., Wang, J.P., Zeng, Q.F., Bai, S.P., Su, Z.W. and Zhang, K.Y. 2016.** Effects of dietary crude protein levels and exogenous protease on performance, nutrient digestibility, trypsin activity and intestinal morphology in broilers. *Livestock Science*, 193: 26–31. <https://doi.org/10.1016/J.LIVSCI.2016.09.002>
- Dongare, P.P., Dhande, S.R. and Kadam, V.J. 2013.** Standardization of Carbon Tetrachloride-Induced Hepatotoxicity In the Rat. *Am. J. PharmTech Res*, 3(5). <http://www.ajptr.com/>
- Donsbough, A.L., Powell, S., Waguespack, A., Bidner, T.D. and Southern, L.L. 2010.** Uric acid, urea, and ammonia concentrations in serum and uric acid concentration in excreta as indicators of amino acid utilization in diets for broilers. *Poultry Science*, 89(2): 287–294. <https://doi.org/10.3382/PS.2009-00401>
- Doskovič, V., Bogosavljevič-Boskovič, S., Pavlovski, Z., Milošević, B., Škrbič, Z., Rakonjac, S. and Petričević, V. 2013.** Enzymes in broiler diets with special reference to protease. *World's Poultry Science Journal*, 69(2): 343–360. <https://doi.org/10.1017/S0043933913000342>
- Elbaz, A.M., El-sheikh, S.E. and Abdel-Maksoud, A. 2023.** Growth performance, nutrient digestibility, antioxidant state, ileal histomorphometry, and cecal ecology of broilers fed on fermented canola meal with and without exogenous enzymes. *Tropical Animal Health and Production*, 55(1): 1–11. <https://doi.org/10.1007/s11250-023-03476-9>
- Embaby, H.E.S. 2011.** Effect of heat treatments on certain antinutrients and in vitro protein digestibility of peanut and sesame seeds. *Food Science and Technology Research*, 17(1): 31–38. <https://doi.org/10.3136/FSTR.17.31>

- Erdaw, M.M., Bhuiyan, M. and Iji, P.A. 2017a.** Partial replacement of commercial soybean meal with raw, full-fat soybean meal supplemented with varying levels of protease in diets of broiler chickens. , 47(1).
- Erdaw, M.M., Bhuiyan, M.M. and Iji, P.A. 2017b.** Response of broiler chicks to non-steam- or steam-pelleted diets containing raw, full-fat soybean meal. : 260–272.
- Erdaw, M.M., Perez-Maldonado, R.A. and Iji, P.A. 2018.** Supplementation of broiler diets with high levels of microbial protease and phytase enables partial replacement of commercial soybean meal with raw, full-fat soybean. *Journal of Animal Physiology and Animal Nutrition*, 102(3): 755–768. <https://doi.org/10.1111/jpn.12876>
- Erdaw, M.M., Wu, S. and Iji, P.A. 2017.** Growth and physiological responses of broiler chickens to diets containing raw, full-fat soybean and supplemented with a high-impact microbial protease. *Asian-Australasian Journal of Animal Sciences*, 30(9): 1303–1313. <https://doi.org/10.5713/ajas.16.0714>
- Freitas, D.M., Vieira, S.L., Angel, C.R., Favero, A. and Maiorka, A. 2011.** Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. *Journal of Applied Poultry Research*, 20(3): 322–334. <https://doi.org/10.3382/JAPR.2010-00295>
- Ghazi, S., Rooke, J.A. and Galbraith, H. 2003.** Improvement of the nutritive value of soybean meal by protease and alpha-galactosidase treatment in broiler cockerels and broiler chicks. *British poultry science*, 44(3): 410–418. <https://doi.org/10.1080/00071660310001598283>
- Ghazi, S., Rooke, J.A., Galbraith, H. and Bedford, M.R. 2002.** The potential for the improvement of the nutritive value of soybean meal by different proteases in broiler chicks and broiler cockerels. *British poultry science*, 43(1): 70–77. <https://doi.org/10.1080/00071660120109935>
- Gilbert, E.R., Wong, E.A. and Webb, K.E. 2008.** Board-invited review: Peptide absorption and utilization: Implications for animal nutrition and health. *Journal of Animal Science*, 86(9): 2135–2155. <https://doi.org/10.2527/JAS.2007-0826>
- Hanauska-Brown, L.A., Dufty, A.M. and Roloff, G.J. 2003.** Blood chemistry, cytology, and body condition in adult Northern Goshawks (*Accipiter Gentilis*). *Journal of Raptor Research*, 37(4): 299–306.
- Hernandes, R. and Cazetta, J.O. 2001.** Método Simples e Acessível para Determinar Amônia Liberada pela Cama Aviária 1 Simple And Accessible Method To Determine Liberated Ammonia from the Broiler Litter. *Revista Brasileira de Zootecnia*, 30(3): 824–829.
- Jiang, Y., Li, L., He, F., Yan, W., Tang, Y., Yang, R., and Zhao, W. 2021.** Highly effective inactivation of anti-nutritional factors (lipoxygenase, urease and trypsin inhibitor) in soybean by radio frequency treatment. *International Journal of Food Science and Technology*, 56(1), 93-102. <https://doi.org/10.1111/ijfs.14605>
- Kaczmarek, S.A., Rogiewicz, A., Mogielnicka, M., Rutkowski, A., Jones, R.O. and Slominski, B.A. 2014.** The effect of protease, amylase, and nonstarch polysaccharide-degrading enzyme supplementation on nutrient utilization and growth performance of broiler chickens fed corn-soybean meal-based diets. *Poultry science*, 93(7): 1745–1753. <https://doi.org/10.3382/PS.2013-03739>
- Kamel, N.F., Naela, Ragaa, M., El-Banna, R.A. and Mohamed, F.F. 2015.** Effects of a Monocomponent Protease on Performance Parameters and Protein Digestibility in Broiler Chickens. *Agriculture and Agricultural Science Procedia*, 6: 216–225. <https://doi.org/10.1016/j.aaspro.2015.08.062>
- Karr-Lilienthal, L.K., Kadzere, C.T., Grieshop, C.M. and Fahey, G.C. 2005.** Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: A review. *Livestock Production Science*, 97(1): 1–12. <https://doi.org/10.1016/J.LIVPRODSCI.2005.01.015>
- Kim, B.G. & Lindemann, M.D. 2007.** A spreadsheet method for experimental animal allotment. *Journal of Animal Science*, 85(Suppl. 2): 112.
- Kim, M., Ingale, S.L., Hosseindoust, A., Choi,**

- Y., Kim, K. and Chae, B. 2021.** Synergistic effect of exogenous multi-enzyme and phytase on growth performance, nutrients digestibility, blood metabolites, intestinal microflora and morphology in broilers fed corn-wheat-soybean meal diets. *Animal Biology*, 34(8): 1365.
- Laborde, C.J., Chapa, A.M., Burleigh, D.W., Salgado, D.J. and Fernandez, J.M. 1995.** Effects of processing and storage on the measurement of nitrogenous compounds in ovine blood. *Small Ruminant Research*, 17(2): 159–166. [https://doi.org/10.1016/0921-4488\(95\)00665-8](https://doi.org/10.1016/0921-4488(95)00665-8)
- Mohammadigheisar, M., Shouldice, V.L., Torrey, S., Widowski, T.M., Ward, N.E. and Kiarie, E.G. 2021.** Growth performance, organ attributes, nutrient and caloric utilization in broiler chickens differing in growth rates when fed a corn-soybean meal diet with multienzyme supplement containing phytase, protease and fiber degrading enzymes. *Poultry science*, 100(9). <https://doi.org/10.1016/J.PSJ.2021.101362>
- Naseem, S. and King, A. 2018.** Ammonia production in poultry houses can affect health of humans, birds, and the environment—techniques for its reduction during poultry production. *Environmental Science and Pollution Research International*, 25(16): 15269–15293.
- Ndazigaruye, G., Kim, D.H., Kang, C.W., Kang, K.R., Joo, Y.J., Lee, S.R. and Lee, K.W. 2019.** Effects of low-protein diets and exogenous protease on growth performance, carcass traits, intestinal morphology, cecal volatile fatty acids and serum parameters in broilers. *Animals: an Open Access Journal from MDPI*, 9(5): 226. <https://doi.org/10.3390/ANI9050226>
- NRC. 1994.** Nutrient requirements of poultry. 9th rev. ed.
- Omar, A.E., Al-Khalaifah, H.S., Ismail, T.A., Abd El-Aziz, R.M., El-Mandrawy, S.A.M., Shalaby, S.I. and Ibrahim, D. 2021.** Performance, Serum Biochemical and Immunological Parameters, and Digestive Enzyme and Intestinal Barrier-Related Gene Expression of Broiler Chickens Fed Fermented Fava Bean By-Products as a Substitute for Conventional Feed. *Frontiers in Veterinary Science*, 8(July): 1–13. <https://doi.org/10.3389/fvets.2021.696841>
- Pacheco, W.J., Stark, C.R., Ferket, P.R. and Brake, J. 2014.** Effects of trypsin inhibitor and particle size of expeller-extracted soybean meal on broiler live performance and weight of gizzard and pancreas. *Poultry Science*, 93(9): 2245–2252. <https://doi.org/10.3382/ps.2014-03986>
- Parlee, S.D., Lentz, S.I., Mori, H. and MacDougald, O.A. 2014.** Quantifying Size and Number of Adipocytes in Adipose Tissue. *Methods in enzymology*, 537: 93. <https://doi.org/10.1016/B978-0-12-411619-1.00006-9>
- Perilla, N.S., Cruz, M.P., De Belalcázar, F. and Diaz, G.J. 1997.** Effect of temperature of wet extrusion on the nutritional value of full-fat soybeans for broiler chickens. *British poultry science*, 38(4): 412–416. <https://doi.org/10.1080/00071669708418011>
- Rehman, Z.U., Kamran, J., El-Hack, M.E.A., Alagawany, M., Bhatti, S.A., Ahmad, G., Saleem, A. et al. 2017.** Influence of low-protein and low-amino acid diets with different sources of protease on performance, carcasses and nitrogen retention of broiler chickens. *Animal Production Science*, 58(9): 1625–1631. <https://doi.org/10.1071/AN16687>
- Ruiz N., De Belalcazar F. 2005** Field observations: trypsin inhibitors in soybean meal are correlated with outbreaks of feed passage in broilers. *Poultry science* 84:70
- Saleh A. A. 2017.** Influence of *Escherichia coli* 6-Phytase supplementation on performance and egg quality in Hi-sex laying hens fed phosphorus deficient diets. *Egyptian Poultry Science*. 37:1105–1117
- Saleh, A.A., Dawood, M.M., Badawi, N.A., Ebeid, T.A., Amber, K.A. and Azzam, M.M. 2020.** Effect of supplemental serine-protease from *Bacillus licheniformis* on growth performance and physiological change of broiler chickens. <http://www.tandfonline.com/action/journalInformation?show=aimsScope&journalCode=taar20#.VsXoziCLRhE>, 48(1): 86–92. <https://doi.org/10.1080/09712119.2020.1732986>
- Saleh, A.A., El-Far, A.H., Abdel-Latif, M.A., Emam, M.A., Ghanem, R. and Abd El-Hamid, H.S. 2018.** Exogenous dietary

- enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. *PLOS ONE*, 13(5): e0198085.
<https://doi.org/10.1371/JOURNAL.PONE.0198085>
- Sorbara, J.O.2009.** Use of a protease to enhance the utilisation of soybean meal amino acids by broilers. *Poultry Science . Abstract 88:221*, Suppl: 68.
- Takács, K., Szabó, E. E., Nagy, A., Cserhalmi, Z., Falusi, J. and Gelencsér, E. 2022.** The effect of radiofrequency heat treatment on trypsin inhibitor activity and in vitro digestibility of soybean varieties (*Glycine max.* (L.) Merr.). *Journal of Food Science and Technology*, 59 (11): 4436-4445.
- Tajudeen, H., Hosseindoust, A., Ha, S.H., Moturi, J., Mun, J.Y., Lee, C.B., Park, J.W. et al.2022.** Effects of dietary level of crude protein and supplementation of protease on performance and gut morphology of broiler chickens. *European Poultry Science*, 86.
<https://doi.org/10.1399/EPS.2022.355>
- Thongprayoon, C., Cheungpasitporn, W. and Kashani, K.2016.** Serum creatinine level, a surrogate of muscle mass, predicts mortality in critically ill patients. *Journal of Thoracic Disease*, 8(5): E305–E311.
<https://doi.org/10.21037/jtd.2016.03.62>
- Walk, C.L., Juntunen, K., Paloheimo, M. and Ledoux, D.R.2019.** Evaluation of novel protease enzymes on growth performance and nutrient digestibility of poultry: enzyme dose response. *Poultry Science*, 98(11): 5525–5532.
<https://doi.org/10.3382/PS/PEZ299>
- Wang, H., Guo, Y. and Shih, J.C.H.2008.** Effects of dietary supplementation of keratinase on growth performance, nitrogen retention and intestinal morphology of broiler chickens fed diets with soybean and cottonseed meals. *Animal Feed Science and Technology*, 140(3–4): 376–384.
<https://doi.org/10.1016/J.ANIFEEDSCI.2007.04.003>
- Wijtten, P.J.A., Hangoor, E., Sparla, J.K.W.M. and Verstegen, M.W.A.2010.** Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. *Poultry Science*, 89(7): 1424–1439.
<https://doi.org/10.3382/ps.2009-00626>
- Zuo, J., Ling, B., Long, L., Li, T., Lahaye, L., Yang, C. and Feng, D. 2015.** Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. *Animal nutrition (Zhongguo xu mu shou yi xue hui)*, 1(4): 276–282.
<https://doi.org/10.1016/J.ANINU.2015.10.003>

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