ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 64 183 Number 5, 2016
http://dx.doi.org/10.11118/actaun201664051645

THE EFFECT OF EXOGENOUS PROTEASE IN BROILER DIETS ON THE APPARENT ILEAL DIGESTIBILITY OF AMINO ACIDS AND ON PROTEASE ACTIVITY IN JEJUNUM

Vojtěch Rada[1], Martina Lichovníková[1], Marian Foltyn[1], Ivo Šafařík[2]

[1] Department of Animal Breeding, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic
[2] Department of Nanobiotechnology, Biology Centre, ISB, CAS, 370 05 České Budějovice, Czech Republic

Abstract

RADA VOJTĚCH, LICHOVNÍKOVÁ MARTINA, FOLTYN MARIAN, ŠAFAŘÍK IVO. 2016. The Effect of Exogenous Protease in Broiler Diets on the Apparent Ileal Digestibility of Amino Acids and on Protease Activity in Jejunum. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 64(5): 1645–1652.

The objective of this study was to evaluate the effect of a mono-component commercial serine protease supplement in broiler diets on apparent ileal amino acid digestibility and protease activity. A total of 150 male (28 d old) ROSS 308 were randomly placed into 30 battery pens and divided into 5 treatment groups with 6 replicates each. The experiment was performed for 7 days. Five dietary treatments were used: 2 standard protein diets without (SP) and with protease (SP + P) formulated 20.7 % CP, 2 lower-protein diets (19.9 % CP) without (LP) and with protease (LP + P) and one lower-protein diet with protease and with doubled rapeseed meal (RSM) content (SP-RSM + P) compared with the other treatments. Lower-protein diets were formulated with a 4 % decrease in the relative CP value compared with the standard protein diet. Enzyme protease was added to the diets at a concentration of 200 ppm (15,000 PROT units per kg). The diets contained 0.3 % Cr_2O_3 to facilitate the estimation of apparent AA digestibility and overall apparent ileal crude protein digestibility. Mono-component protease had no effect on apparent ileal AA digestibility or jejunum protease activity if diets contained the same level of RSM. The supplement of exogenous protease did not affect ($P > 0.05$) the apparent ileal AA digestibility coefficients if a higher RSM level was used. The CP level influenced ($P < 0.05$) only the coefficients of the apparent ileal AA digestibility of Pro and Arg. The RSM level ($P < 0.01$) had significant effects on protease activity in the jejunum.

Keywords: chicken, crude protein, methionine, glutamic acid, rapeseed meal

INTRODUCTION

The improvement of feed efficiency is an important issue in animal nutrition because of the need to reduce environmental pollution from farm animals and to decrease production costs. Feedstuffs contain certain compounds that animals cannot digest or that interfere with the animals' digestive system. A frequent reason for these problems is that the animals are unable to produce the necessary enzymes to degrade the compounds (Khattak *et al.*, 2006). To reduce total nutrient excretion, which results from incomplete digestion, is one of the major targets of European Directive 2001/81/EC (National Emission Ceilings). In recent decades, much research has been performed in the study of chicken nutrition to investigate the use of exogenous enzymes to improve nutrient utilization (Campbell *et al.*, 1992; Leeson *et al.*, 1996; Leeson *et al.*, 2005; Seskeviciene *et al.*, 1999; Smits *et al.*, 1996) and many commercial enzyme products are currently available for use in chicken nutrition. Phytases are already well established in chicken diets, and research is now focusing more on the other enzymes.

Several previous studies have reported that a wide range of endogenous proteases are synthesized and released in the gastrointestinal tract. These proteases are considered sufficient to optimize feed protein utilization (Le Heurou-Luron *et al*., 1993; Nir *et al*., 1993). Nevertheless, a certain amount of protein passes through the gastrointestinal tract without being completely digested (Wang and Parsons, 1998; Lemme *et al*., 2004). The protein that is excreted by chickens as a result of incomplete protein digestibility presents an opportunity for the use of specific exogenous proteases. However, the results of studies conducted with exogenous proteases are inconsistent and variable because of the diversity of feed ingredients and of the types and definitions of protease itself and because of differences in methodology (Angel *et al.* 2011; Fru-Nji *et al*. 2011; Ghazi *et al.* 2003; Marsman *et al*. 1997; McNab *et al*. 1996; Naveed *et al*. 1998; Simbaya *et al.* 1996). Ghazi *et al*. (2002) have demonstrated a positive effect of feed pretreatment by protease in both *in vitro* and *in vivo* studies for diets that were deficient or marginal in amino acids (AAs). Additionally, Ghazi *et al*. (2003) have reported large differences in true N digestibility and in ME in broilers when different protease sources were used. In contrast, Mahagna *et al*. (1995) have found that a higher level of protease supplementation depressed the endogenous production of proteases in broilers.

Currently, a new serine protease expressed in *Bacillus licheniformis* was introduced into the poultry industry. This feed protease is claimed to act through the solubilization and hydrolysis of dietary proteins and to have a non-specific mode of action on a broad range of dietary proteins (Fru-Nji *et al*. 2011).

The objective of this study was to evaluate the effect of a mono-component serine protease supplement in broiler diets on apparent ileal amino acid digestibility and protease activity in jejunum while using two levels of crude protein in the diets. The effect of protease on amino acids digestibility from the diet with higher content of rapeseed meal was observed too.

MATERIALS AND METHODS

Management and Diets of Birds

The enzyme used in this study is described as a purified mono-component serine protease. This protease is expressed by a *Bacillus licheniformis* strain that contains transcribed genes from *Nocardiopsis prasina*. For the *in vivo* studies, a heat-stable formulated product containing 75,000 PROT units/g was used. One PROT unit is defined as the amount of enzyme that releases 1 µmol of *p*-nitroaniline from 1 µM of substrate (Suc-Ala-Ala-Pro-Phe-*N*-succinyl-Ala-Ala-Pro-Phe--*p*-nitroanilide) per minute at pH 9.0 and 37 °C. The 7-d experiment was conducted at Mendel University in Brno in accordance with the official Czech norms for experiments with live animals. A total of 150 male ROSS 308 broiler chickens (28 d old) were used in the experiment. Broilers of similar weight (1550 ± 50 g) were selected and randomly placed into one of the 30 battery pens (5 broilers per pen) in an environmentally controlled room. The lighting was controlled to provide a photoperiod of 18L:6D, and the room temperature was adapted according to the age-specific requirements of the birds. The birds were allowed *ad libitum* access to feed and water. Nipple drinkers with cups were used, three in each cage. Feed was mash form fed twice a day by hand. Five dietary treatments (Tab. I) based on wheat, corn and soybean meal were used, with 6 replicates for each treatment. The first 2 diets contained a standard level of protein (20.7 % CP). In the first treatment (SP), the diet was fed without protease supplementation. In the second treatment (SP + P), the standard level of protein was supplemented with 15,000 PROT units per kg diet. The level of CP in the third, fourth and fifth diets was reduced by 4 % compared with SP to formulate a lower-protein diet (LP; 19.9 % CP). The 4 % SP reduction was based on the recommendation of the producer of the exogenous protease used in the study. In the third treatment (LP), this diet was fed without protease supplementation. In the fourth treatment (LP + P), this diet was fed with the supplementation of 15,000 PROT units per kg diet. In the fifth treatment (SP-RSM + P), the rapeseed meal (RSM) content was increased twofold (80 g of RSM per kg of the diet) compared with the SP diet, and the diet was additionally supplemented with

I: *Study treatments*

Diet	CP level	RSM level[1]	Protease
SP	standard	4	no
SP + P	standard	4	yes
LP	lower	4	no
LP + P	lower	4	yes
SP-RSM + P	standard	8	yes

[1]RSM-rapeseed meal in the diets (%)

15,000 PROT units per kg. The protease was added to the treatments (SP + P, LP + P, SP-RSM + P) at the same level (15,000 PROT units per kg of the diet) according to the recommendations for grower diets. All diets were optimized to the same ME level (12.7 MJ/kg feed). The diets differed in their CP level, and in the fifth dietary treatment there was higher content of RSM. Chromium oxide (Cr_2O_3) was added to the diets as an indigestible marker at a concentration of 3 g per kg feed (3,000 ppm). The composition of the experimental diets is shown in Tab. II.

II: *Composition of the diets (g/kg)*

Ingredient	SP	SP + P	LP	LP + P	SP-RSM + P
Wheat	298.5	298.0	323.7	323.5	294.1
Maize	300	300	300	300	300
Soybean meal	281.2	282.3	259.8	259.8	242.7
Rapeseed meal (RSM)	40	40	40	40	80
Soybean oil	44.2	43.4	40.5	40.5	48.2
Sodium Chloride	2.15	2.15	2.14	2.14	2.13
Sodium sulphate	1.92	1.92	1.92	1.92	1.86
DL-Methionine	2.49	2.51	2.29	2.29	2.18
Lysine HCl	2.56	2.56	2.56	2.56	2.47
L-Threonine	0.75	0.76	0.7	0.7	0.59
Limestone	14.13	14.13	14.2	14.2	13.69
Monocalcium phosphate	7.40	7.39	7.54	7.54	7.23
Phytase	0.9	0.9	0.9	0.9	0.9
Xylanase	0.5	0.5	0.5	0.5	0.5
Vitamin-mineral mix[1]	3.00	3.00	3.00	3.00	3.00
Chromium oxide	0.3	0.3	0.3	0.3	0.3
Protease	**0**	**0.2**	**0**	**0.2**	**0.2**
Nutritional value, %					
ME, MJ/kg	12.7	12.7	12.7	12.7	12.7
CP	20.7	20.7	19.9	19.9	20.1
Lysine	1.25	1.25	1.20	1.20	1.20
Methionine + Cystine	0.93	0.93	0.88	0.88	0.89
Threonine	0.83	0.83	0.79	0.79	0.80
Arginine	1.32	1.33	1.26	1.26	1.27
Digestible Lysine	1.11	1.12	1.11	1.11	1.11
Digestible Methionine + Cystine	0.82	0.86	0.80	0.82	0.82
Digestible Threonine	0.71	0.74	0.70	0.71	0.71
Digestible Arginine	1.17	1.23	1.16	1.17	1.18
Ca	0.76	0.76	0.76	0.76	0.76
Available P	0.40	0.40	0.40	0.40	0.40
Na	0.16	0.16	0.16	0.16	0.16

[1] Vitamin, mineral, and additive contibutions per kilogram of feed: Vit. A: 250,000 i.u., Vit. D3: 40,000 i.u.; Vit. E (alfa tocopherol): 700 mg; Vit. K3: 30 mg; Vit. B1: 30 mg; Vit. B2: 60 mg; Vit. B6: 25 mg; Vit. B12: 0.2 mg; Niacinamid: 210 mg; Cholin chloride: 6,200 mg; DL-methionin: 20 g; L-lysine: 14 g; Ca: 200 g; P: 48 g; Na: 15 g; Fe: 880 mg; Cu: 100 mg; Zn: 740 mg; Mn: 1 240 mg; Co: 4.5 mg; I: 5 mg; Se: 1.4 mg

Chemical analyses

The dry matter was determined by drying the samples (content of ileum and diets) at 103 ± 2 °C for four hours. To measure the content of amino acids in the diets and digesta, oxidative acid hydrolysis was used (HCl, c = 6 mol/l). The chromatographic analysis of the hydrolysate samples was performed in an AAA 400 analyzer (Ingos, Prague, Czech Republic) using Na-citrate buffers and ninhydrin detection to determine the amounts of specific amino acids. The N content was analyzed using the Kjeldahl method.

Apparent Ileal AA Digestibility

At the end of the experiment, after 7 days of feeding the experimental diets, the broilers were killed by decapitation (at the age of 35 d) and dissected to obtain the digesta from the last third of the ileum, but 4 cm from the ileocecal junction. The collected digesta were stored at −30 °C (one pooled sample of 5 chickens). Samples of digesta were lyophilized, ground and analyzed for amino acids, CP, dry matter and Cr_2O_3. The samples of the feed and ileal digesta were treated by oxidative acid hydrolysis with HCl (6 mol.l^{-1}). The chromatographic analysis of the hydrolyzate samples was performed with an AAA 400 analyzer (f. Ingos, Prague) using Na-citrate buffers and ninhydrin detection to determine the amounts of certain amino acids.

The apparent ileal AA digestibility was calculated with the following formula:

$$\text{Apparent Ileal AA Digestibility} = 100 - (100 \times I_d \times AA_{dc} / I_{dc} \times AA_d)^* \ (\%)$$

* content of indicator in the diet (I_d),
content of AA in the digesta (AA_{dc}),
content of indicator in the digesta (I_{dc}),
content of AA in the diet (AA_d).

In the experiment, estimates were obtained of the apparent ileal AA digestibility, the digestibility of essential and non-essential AAs and the overall apparent ileal crude protein digestibility. The results were expressed as coefficient of digestibility.

III: *Coefficients of apparent ileal AA digestibility at 35 d of age*

	n	SP	SP + P	LP	LP + P	SP-RSM + P	SEM	CP level	Protease
Asparatic acid	6	0.776	0.794	0.769	0.759	0.794	0.023	NS	NS
Threonine	6	0.713	0.728	0.729	0.725	0.766	0.034	NS	NS
Serine	6	0.750	0.778	0.773	0.773	0.801	0.030	NS	NS
Glutamic acid	6	**0.798^{a}**	**0.841^{ab}**	**0.843^{ab}**	**0.829^{ab}**	**0.862^{b}**	0.025	NS	NS
Proline	6	**0.898^{a}**	**0.881^{a}**	**0.936^{b}**	**0.927^{ab}**	**0.919^{ab}**	0.013	< 0.001	NS
Glycine	6	0.741	0.757	0.748	0.736	0.770	0.029	NS	NS
Alanine	6	0.766	0.777	0.776	0.768	0.796	0.028	NS	NS
Valine	6	0.748	0.770	0.764	0.760	0.792	0.081	NS	NS
Methionine	6	**0.816^{a}**	**0.858^{ab}**	**0.843^{ab}**	**0.885^{ab}**	**0.896^{b}**	0.035	NS	NS
Isoleucine	6	0.780	0.813	0.798	0.803	0.836	0.032	NS	NS
Leucine	6	0.776	0.805	0.799	0.787	0.816	0.027	NS	NS
Tyrosine	6	0.801	0.822	0.814	0.815	0.826	0.027	NS	NS
Phenylalanine	6	0.797	0.817	0.801	0.796	0.819	0.024	NS	NS
Histidine	6	0.814	0.811	0.794	0.782	0.802	0.020	NS	NS
Lysine	6	0.856	0.856	0.836	0.820	0.827	0.021	NS	NS
Arginine	6	**0.847^{b}**	**0.863^{b}**	**0.798^{a}**	**0.792^{a}**	**0.837^{ab}**	0.022	< 0.001	NS
Σ essential AA	6	0.777	0.805	0.796	0.791	0.818	0.025	NS	NS
Σ non-essential AA	6	0.777	0.801	0.810	0.798	0.822	0.026	NS	NS
Σ total AA	6	0.787	0.803	0.804	0.795	0.820	0.025	NS	NS
N	6	0.730	0.756	0.774	0.764	0.766	0.032	NS	NS

Different superscripts (a, b) indicate statistical significant difference between groups (P < 0.05)

Protease Activity

The digesta to be analyzed for protease activity were collected from the section of the jejunum between the duodenum and Meckel's diverticulum. Samples of digesta were diluted 10× with ice-cold PBS (pH 7.0) based on the sample weight and homogenized for 10 min in a refrigerator. The samples were then centrifuged at 1,500 × g for 10 min at 4 °C. The supernatant was transferred to Eppendorf tubes and stored at –30 °C for enzyme assays. A modification of the method of Lynn and Clevette-Radford (1984) was used to determine jejunum protease activity. A 1 % solution of azocasein (Sigma-Aldrich) in 1 ml of 0.1 M pH 7 phosphate buffer was digested with the supernatant (digesta sample) at 37 °C for 120 min. The reaction was stopped with trichloroacetic acid (1.5 ml) and clarified by centrifugation, and the absorbance at 366 nm (A_{366nm}) of the supernatant was recorded in a 1 cm cell. The protease activity was expressed as absorbance.

Statistical Analysis

Data were subjected to two-way factorial ANOVA (CP level, protease). The significance of differences between means was determined by LSD test and differences were considered significant at $P < 0.05$. Statistical analyses were performed using the Unistat 6.5.

RESULTS

Apparent Ileal AA Digestibility

The results for apparent ileal AAs digestibility are shown in Tab. III. The addition of exogenous protease had no significant effects ($P > 0.05$) on the apparent ileal AA digestibility coefficients or the apparent ileal nitrogen coefficient. The CP level of the diets had significant ($P < 0.001$) influence on the apparent ileal digestibility of Pro and Arg. In the chickens fed the diet with a standard protein level, the addition of protease did not significantly increase the digestibility of any of the observed AAs. The digestibility of the AAs in the lower-protein diets was similar to that in the standard protein diets. No significant differences were observed between the LP and LP + P treatments. The addition of protease slightly decreased the digestibility of most of the AAs (with the exception of Ser, Met, Ileu and Tyr) in the LP diets (LP in comparison with LP + P), but the difference was not significant. The digestibility of Arg significantly ($P < 0.05$) decreased in the LP and LP + P treatments in comparison with the SP and SP + P treatments. The digestibility of Pro was significantly ($P < 0.05$) lower in the SP + P and SP treatments than in the LP treatment.

The exogenous protease supplemented in the standard-CP diet (SP-RSM + P) but with a higher content of RSM (8 %) had a slight positive effect (no significant) on all AAs digestibility except His and Lys in comparison with the diet with the standard CP level and lower RSM (4 %) content (SP). Digestibility of Met and Glu were even significantly higher in SP-RSM + P than in SP ($P < 0.05$). It follows from these findings that the addition of protease slightly improved the digestibility of AAs if the RSM content in the broiler diets is higher.

Protease activity

The effects of protease addition to broiler diets on protease activity in jejunum are shown in Tab. IV. The addition of exogenous protease and CP level had no significant effect on protease activity. The protease activity in the SP-RSM + P treatment was significantly lower ($P < 0.05$) than that in the SP, SP + P and LP treatments. The higher level of RSM (4 vs. 8 %) had negative effect on protease activity.

IV: *The jejunum protease activity*

	Mean	SE
SP	0.727^{b}	0.027
SP + P	0.764^{b}	0.016
LP	0.716^{b}	0.027
LP + P	0.679^{ab}	0.016
SP-RSM + P	0.617^{a}	0.019
CP level	NS	
Protease	NS	

Different superscripts (a, b) indicate statistical significant difference between groups ($P < 0.05$)

DISCUSSION

Currently, a few enzymes are already in use and established as a component of broiler diets. The most commonly used enzyme is phytase, followed by xylanase and amylase. However, this is not the case for protease, and the results of studies conducted with mono-component protease are frequently inconsistent. In this context, the effects of exogenous protease on broilers are not clear due to differences in the types of proteases tested and dissimilar methodology as well as differences in the composition of the negative control diet. These considerations can partially explain the conflicting and highly variable results that have been reported (Ghazi *et al.* 2002; McNab *et al.* 1996; Marsman *et al.* 1997; Olukosi *et al.* 2011; Walk *et al.* 2011).

Crude protein and AAs are costly nutrients that are obtained from dietary ingredients (with the exception of the synthetic form of certain AAs). They are directly influenced by proteases. CP degradation by gastric and pancreatic secretions is generally moderate (Moran *et al.* 1982). The digestibility of CP is variable in terms of the source of CP (types of feed ingredients and their variability). As a result, a certain amount of CP passes through the gastrointestinal tract in undigested or incompletely digested form (Lemme *et al.* 2004). Under these circumstances, the use of exogenous protease could

increase CP utilization and help control feeding costs.

Most studies that have addressed the use of exogenous protease have investigated enzymes supplied in the form of cocktails. In such studies, several enzymes are tested together. Accordingly, the effect of the enzyme preparation cannot always be attributed to the addition of a specific enzyme, particularly because few studies have addressed the use of mono-component protease. In the present study, mono-component protease was used individually without other enzymes to supplement broiler diets with standard and lower levels of protein. In the study, determinations of the digestibility of AAs found no effect of protease addition. Similar results have been reported by Ghazi *et al*. (2003), who found no effect of protease addition on true nitrogen digestibility using proteases from *Aspergillus niger* and *Bacillus subtilis*. In contrast, Angel *et al*. (2011) have shown that the addition of a protease extracted from *Bacillus licheniformis* had positive effects on the digestibility of CP and certain AAs (Arg, Ile, Lys, Thr, His, Asp, Cys and Ser) in lower-protein diets compared with a standard protein diet or a lower-protein diet without protease supplementation. Angel *et al*. (2011) found that the digestibility of Leu, Phe, Ala, Glu, Tyr, Pro and Gly was not affected by exogenous protease. These results are consistent with the results of the present study. The same study also found that the protease had a significant positive effect on AA digestibility at doses of 15,000 PROT units/kg or greater. Freitas *et al*. (2011) used the same protease as that added in the present study and in the Angel *et al*. (2011) study. They also found a positive effect of protease addition on CP digestibility and even on fat digestibility. Additionally, Fru-Nji *et al*. (2011) found increases in CP digestibility and fat digestibility as a result of the improved energy digestibility resulting from the addition of protease to broiler diets. They also concluded that the effect of exogenous protease appears to be more pronounced in feeds with lower nutrient digestibility. The parallel effect of supplemented protease on both CP digestibility and fat digestibility appears obvious from these reports (Angel *et al*. 2011; Fru-Nji *et al*. 2011). How the protease could improve fat digestibility is currently unknown. Nevertheless, it has been hypothesized that some undigested protein may bind to free fatty acids and interfere with their absorption (Sklan *et al*. 1975).

The examination of the effect of exogenous protease on ileal protease activity indicated that supplementation with protease did not affect ileal protease activity in any treatment except SP-RSM + P. The endogenous production of protease in the chicken gastrointestinal tract is considered sufficient for feed protein utilization (Le Heurou-Luron *et al*. 1993; Nir *et al*. 1993). In this context, the use of exogenous proteases may not increase ileal protease activity or may substitute for the bird's endogenous protease. The effect of the exogenous proteases on ileal protease activity depends on the dietary protein source (Makkink *et al*. 1994; Marsman *et al*. 1997; Yu *et al*. 2002) and type (Ghazi *et al*. 2002) or on the stability of the protease. For instance, bromelain (a plant protease) showed hydrolytic capability *in vitro*, but did not demonstrate the same level of increased enzymatic activity in the gastrointestinal tract in practical broiler feeding (Yu *et al*. 2002). Bhat and Hazlewood (2001) showed that every enzyme has to build an effective enzyme-substrate complex to achieve the correct mode of action and release its products after the reaction is complete. For the endogenous proteases, the specificity of the peptide bond is a major determinant of the rate of protein hydrolysis and of the quantity of peptides and AAs released and made available for adsorption (Moran, 1982). The peptide bond affinities of the serine protease used in this study have not yet been established (Angel *et al*. 2011). Other research focused on protease effect at using greater reduction of CP level would be necessary to confirm its positive effect.

CONCLUSION

Mono-component protease had no significant effect on apparent ileal AAs digestibility or protease activity in jejunum at feeding diets containing the level of CP 19.9 and 20.7 %. The exogenous protease supplement had a slight positive effect on the apparent ileal AAs coefficients of digestibility when a higher RSM level was used, except Lysine and Histidine, 4 vs. 8 %. The exogenous protease significantly increased Methionine and Glutamic acid ileal digestibility when higher RSM level was used ($P < 0.05$). The CP level only influenced ($P < 0.05$) the coefficients of apparent ileal AA digestibility of Proline and Arginine. The higher level of RSM (4 vs. 8 %) significantly decreased ($P < 0.05$) protease activity in jejunum.

Acknowledgements

This work was supported by the Internal Grant Agency at the Mendel University in Brno, Faculty of Agronomy, under Grant IP 8/2015.

REFERENCES

ANGEL, C.R., SAYLOR, W., VIEIRA, S. L. and WARD, N. 2011. Effect of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. *Poult. Sci.,* 90: 2281–2286.

BHAT, M. K. and HAZLEWOOD, G. P. 2001. *Enzymology and other characteristics of cellulases and xylanases.* New York, NY: CABI Publishing.

CAMPBELL, G. L. and BEDFORD, M. R. 1992. Enzyme application for monogastric feeds: a review. *Can. J. Anim. Sci.,* 72: 449–466.

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. *J. Appl. Poult. Res.,* 20: 347–352.

FRU-NJI, F., KLUENTER, A. M., FISCHER, M. and PONTOPPIDAN, K. 2011. A feed serine protease improves broiler performance and increases protein and energy digestibility. *J. Poult. Sci.,* 48: 239–246.

GHAZI, S., ROOKE, J. A., GALBRAITH, H. and BEDFORD, M. R. 2002. The potential for the improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks and broiler cockerels. *Br. Poult. Sci.,* 43: 70–77.

GHAZI, S., ROOKE, J. A. and GALBRAITH, H. 2003. Improvement of the nutritive value of soybean meal by protease and α-galactosidase treatment in broiler cockerels and broiler chicks. *Br. Poult. Sci.,* 44: 410–418.

KHATTAK, F. M., PASHA, T. N., HAYAT, Z. and MAHMUD, A. 2006. Enzymes in poultry nutrition. *J. Anim. Plant Sci.,* 16: 1–7.

LE HEUROU-LURON, I., LHOSTE, E., WICKER-PLANQUARL, C., DAKKA, N., TOULLEC, R., CORRING, T., GUILLOTEAU P. and PUIGSERVER, A. 1993. Molecular aspects of enzyme synthesis in the exocrine pancreas with emphasis on development and nutritional regulation. *Proceeding of the Nutrition Society,* 52: 301–313.

LEESON, S. and SUMMERS, J. D. 2005. *Commercial Poultry Nutrition.* 3rd ed. Guelph: University Books.

LEESON, S., CASTON, L. J. and YUNGBLUT, D. 1996. Adding Roxazyme to wheat diets of chickens and turkey broilers. *J. Appl. Poult. Res.,* 5: 167–172.

LEMME, A., RAVINDRAN, V. and BRYDEN, W. L. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. *Worlds Poult. Sci. J.,* 60: 423–437.

LYNN, K. R. and CLEVETTE-RADFORD, N. A. 1984. Purification and characterization of Hevain, a serine protease from Hevea Brasiliensis. *Phytochemistry,* 23: 963–964.

MAHAGNA, M., NIR, I., LARBIER, M. and NITSAN, Z. 1995. Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzymes activities and digestibility of nutrients in young meat-type chickens. *Reprod. Nutr. Dev.,* 35: 201–212.

MAKKINK, C. A., BERNTSEN, P. J. M., OP DEN KAMP, B. M. L., KEMP, B. and VERSTEGEN, M. W. A. 1994. Gastric protein breakdown and pancreatic enzyme activities in response to two different dietary protein sources in new weaned pigs. *J. Anim. Sci.,* 72: 2843–2850.

MARSMAN, G. J., GRUPPEN, H., VAN DER POEL, A. F., KWAKKEL, R. P., VERSTEGEN, M. W. and VORAGEN, A. G. 1997. The effect of thermal processing and enzyme treatments of soybean meal on growth performance, ileal nutrient digestibilities, and chyme characteristics in broiler chicks. *Poult. Sci.,* 76: 864–872.

MCNAB, J. M., BERNARD, K., KNOX, A.I., MCNEILL, L. and BEDFORD, M. R. 1996. Effect of dietary enzyme addition on digestibility of nitrogen and energy metabolism in young turkeys, broiler chickens and adult cockerels. *Br. Poult. Sci.,* 37: 65–66 abstract.

MORAN, E. T. J. 1982. *Comparative Nutrition of Fowl and Swine: The Gastrointestinal Systems.* Guelph: Office for Educational Practice.

NAVEED, A., ACAMOVIC, T. and BEDFORD, M. R. 1998. Effect of enzyme supplementation of UK-known Lupinis albus on growth performance in broiler chickens. *Br. Poult. Sci.,* 39: 36–37.

NIR, I., NITSAN, Z. and MAHAGNA, M. 1993. Comparative growth and development of the digestive organs and of some enzymes in broiler and egg type chicks after hatching. *Br. Poult. Sci.,* 34: 523–532.

OLUKOSI, O. A., COWIESON, A. J. and ADEOLA, O. 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually and in combination in broilers. *Poult. Sci.,* 86: 77–86.

SESKEVICIENCE, J., JEROCH, H., DÄNICKE, S., GRUZAUSKAS, R., VÖLKER, L. and BROZ, J. 1999. Feeding value of wheat and wheat-based diets with different content of soluble pentosans when fed to broiler chickens without or with enzyme supplementation. *Arch. Geflügelkd.,* 63: 129–132.

SIMBAYA, J., SLOMMINSKI, B. A., GUENTER, W., MORGAN, A. and CAMPBELL, L. D. 1996. The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry. *Anim. Feed Sci. Technol.,* 61: 219–234.

SKLAN, D., HURWITZ, S., BUDOWSKI, P. and ASCARELLI, I. 1975. Fat digestion and absorption in chicks fed raw or heated soybean meal. *J. Nutr.,* 63: 57–63.

SMITS, C. H. M. and ANNISON, G. 1996. Non-starch plant polysaccharide in broiler nutrition towards a physiologically valid approach to their determination. *Worlds Poult. Sci. J.,* 52: 203–221.

YU, B., LEE, T. T. T. and CHLOU, P. W. S. 2002. Effects of sources of protein and enzyme supplementation

on protein digestibility and chyme characteristic in broilers. *Br. Poult. Sci.*, 43: 424–431.

WALK, C. L., COWIESON, A. J., REMUS, J. C., NOVAK, C. L. and MCELROY, A. P. 2011. Effects of dietary enzymes on performance and intestinal goblet cell number of broilers exposed to a live coccidian oocyst vaccine. *Poult. Sci.*, 90: 91–98.

WANG, X. and PARSONS, C. M. 1998. Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. *Poult. Sci.*, 77: 834–841.

Contact information

Martina Lichovníková: lichovmartina@gmail.com