









Original scientific paper

EFFECTS OF PROTEASE AND SEX ON SKIN, BONE AND MUSCLE YIELDS AND PERCENTAGES OF MAJOR PRIMAL CUTS IN BROILERS



ISSN 2466-4774

<https://www.contagri.info/>


VLADIMIR DOSKOVIĆ ^{1*}, SNEŽANA BOGOSAVLJEVIĆ-BOŠKOVIĆ ¹, ZDENKA ŠKRBIĆ ², MILOŠ LUKIĆ ², BOŽIDAR MILOŠEVIĆ ¹,
³, SIMEON RAKONJAC ¹, VESELIN PETRIČEVIĆ ², SINIŠA BJEDOV ⁴

¹University of Kragujevac, Faculty of Agronomy Čačak, Čačak, Serbia

²Institute for Animal Husbandry, Belgrade-Zemun, Serbia

³University of Pristina, Faculty of Agriculture, Kosovska Mitrovica-Zubin Potok-Lešak, Lešak, Serbia

⁴University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

*Corresponding author: vladosko@kg.ac.rs

Submitted: 05.10.2023.

Accepted: 01.11.2023.

SUMMARY

This study aimed to assess the effects of different dietary levels of the protease enzyme and sex on skin, bone and muscle yields and percentages of breast, drumsticks and thighs as the most valuable carcass parts of broilers. A total of 300 one-day-old, mixed-sex broiler chickens (Cobb 500) were randomly divided into 3 dietary treatments with different levels of protease supplementation (group C: a standard diet without Ronozyme ProAct; group E-I: a diet with 200 mg/kg Ronozyme ProAct and a 4% crude protein reduction compared to the standard diet; and group E-II: a diet with 300mg/kg Ronozyme ProAct and a 6% crude protein reduction compared to the standard diet). All diets were maize- and soybean meal-based. Feed and water were provided ad libitum throughout the 49-day feeding trial. The results obtained indicate that the dietary treatments had no significant effect on skin, bone and muscle yields and percentages of the drumsticks, thighs and breast ($P > 0.05$), except for the breast muscle weight in the female chickens (especially between the E-I and E-II groups), and the drumstick bone percentage in the males between the C and E-2 groups ($P < 0.05$). In all three groups, the male chickens exhibited higher weights of skin, muscle and bone in breast, drumsticks and thighs than the female chickens, and the differences were not significant only in the skin weight of drumsticks and thighs ($P > 0.05$). The females had a higher yield of muscle tissue in breast and drumsticks, a higher percentage of skin in drumsticks and thighs, and a lower percentage of bone in drumsticks and thighs than the males ($P < 0.05$). Major differences resulted from the effect of broiler sex, whereas the dietary treatments containing 3 levels of protease supplementation had a very small effect on the yields and percentages of skin, bone and muscle in breast, drumsticks and thighs.

Key words:

broilers, protease, sex, yields and percentages of skin, bone and muscle

INTRODUCTION

Dietary protein is an essential nutrient that is vital for supporting the lives of both animals and humans. To ensure consistency in nutrition, importance should be given to the ingredients used, the rate of nutrient digestion and absorption, and amino acid utilization. In poultry nutrition, protein is largely provided by soybean meal, but soybean

anti-nutrients such as trypsin inhibitors pose a serious concern as they prevent mineral absorption and limit the use of soybean in animal feeds (Park et al. 2020), which is successfully overcome by heating treatments (Anozie et al. 2018).

Supplementing exogenous enzymes in broiler diets has many essential functions such as improving the digestion and utilisation of certain nutrients, reducing the excretion of nitrogen and ammonia to the environment, and improving the production performance of broilers, while not changing the quality and yield of carcasses and their main cuts (Dosković et al. 2020, 2022, 2023; Jabbar et al. 2021; Li et al. 2023).

Exogenous protease is added to broiler diets to help digest protein into smaller peptides and amino acids (Adebiyi & Olukosi, 2015; Matkawala et al. 2021).

The interaction between diets and sex can be of great importance to determining the meat quality characteristics of broilers. Therefore, this study aimed to evaluate the effects of different levels of the enzyme protease in broiler diets and sex, and their interactions on the skin, bone and muscle yields and percentages of breast, drumsticks and thighs as the most valuable parts of the carcass.

MATERIAL AND METHODS

A total of 300 one-day-old, mixed-sex broiler chickens (Cobb 500) were randomly divided into 3 dietary treatments (groups). Each group included four replicates, each of 25 chicks. The diets contained 3 levels of protease supplementation (group C: a standard diet without Ronozyme ProAct; group E-I: a diet with 200 mg/kg Ronozyme ProAct and a 4% crude protein reduction compared to the standard diet; and group E-II: a diet with 300mg/kg Ronozyme ProAct and a 6% crude protein reduction compared to the standard diet). The birds were fed a corn-soybean meal-based starter diet (22 % crude protein, 0–21 days of age), a grower diet (19 % crude protein, 22–35 days of age), and a finisher diet (17 % crude protein, 36–49 days of age). Feed ingredients (used across feeding stages and experimental groups) and the nutritional value of the experimental diets are presented in Table 1. Feed and water were provided *ad libitum* throughout the experimental period of 49 days. All the chicks were kept under the same management, hygienic and environmental conditions.

Table 1. Ingredients and nutritional value of experimental diets for broiler chickens

Ingredient (%)	Starter stage (1 to 21 d)			Grower stage (22 to 42 d)			Finisher stage (43 to 63 d)			
	Treatments	C	E-I	E-II	C	E-I	E-II	C	E-I	E-II
Maize		52.49	54.92	56.26	63.13	65.28	66.34	68.62	70.60	71.59
Soybean meal		22.24	19.79	18.44	13.00	10.85	9.78	9.10	7.10	6.10
Soybean groats		18.50	18.50	18.50	17.00	17.00	17.00	15.40	15.40	15.40
Feeding yeast		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine (78%)		0.10	0.10	0.10	0.20	0.20	0.20	0.23	0.23	0.23
DL-Methionine (99%)		0.22	0.22	0.22	0.30	0.30	0.30	0.30	0.30	0.30
Limestone		1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Monocalcium phosphate		1.30	1.30	1.30	1.20	1.20	1.20	1.20	1.20	1.20
Salt		0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Calcium formate (30.5%)		0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Captex T		0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix		1	1	1	1	1	1	1	1	1
Protease		0.00	0.02	0.03	0.00	0.02	0.03	0.00	0.02	0.03
Calculated composition										
ME (kcal/kg)		3.081	3.100	3.112	3.157	3.174	3.183	3.181	3.198	3.207
Crude proteins (%)		22.59	21.72	21.24	18.99	18.22	17.84	17.16	16.45	16.09
Crude fats (%)		5.59	5.55	5.70	5.67	5.73	5.76	5.55	5.61	5.64
Ca (%)		0.96	0.95	0.95	0.91	0.91	0.90	0.90	0.89	0.89
Total P (%)		0.73	0.72	0.72	0.68	0.67	0.67	0.66	0.65	0.65
Available P (%)		0.44	0.44	0.43	0.40	0.40	0.40	0.39	0.39	0.39
Total lysine (%)		1.33	1.27	1.24	1.15	1.10	1.08	1.05	1.00	0.98
Methionine+cystine (%)		0.92	0.90	0.89	0.91	0.89	0.88	0.86	0.84	0.83

Legend: Treatments: C - control group; a standard broiler diet, without protease; E-I – a diet with a 4% reduction in crude protein level as compared to the control group, and 0.02% protease supplementation; E-II – a diet with a 6% reduction in crude protein level as compared to the control group, and 0.03% protease supplementation

At 49 days of age, a total of 24 birds were randomly selected from each dietary treatment group (six birds per replicate, three males and three females) and slaughtered. After cooling, the carcasses were cut into the major parts – breast, drumsticks and thighs – which were weighed and dissected into muscle, skin and bone, whose weights were also subsequently determined.

The percentages of skin, muscle and bones in the breast, drumsticks and thighs were calculated according to the weights of the skin, bone and muscle and the weights of the breast, drumsticks and thighs.

The results obtained were analysed using the Stat Soft Inc. Statistica for Windows (Version 7.0., 2006). A two-factor (dietary treatments and sex) analysis of variance and the LSD test were used to compare the treatment means ($P < 0.05$), and significant differences were indicated by different superscript letters.

RESULTS AND DISCUSSION

The results of the weights of skin, bone and muscle in the breast, drumsticks and thighs of chickens at 49 days of age are presented in Table 2.

Table 2. Weights of skin, muscle and bone in the breast, drumsticks and thighs of broilers (g)

Treatment		Breast			Drumsticks			Thighs			
Groups	Sex	skin	muscle	bone	skin	muscle	bone	skin	muscle	bone	
C	Male	\bar{X}	70.91 ^{ab}	670.68 ^a	114.71 ^a	19.60	105.25 ^a	45.60 ^a	23.64	142.99 ^a	31.66 ^a
		Sd	15.39	43.45	12.93	3.77	12.48	5.53	3.13	18.56	4.79
	Female	\bar{X}	55.64 ^c	595.54 ^{bc}	89.88 ^b	17.34	89.87 ^{bc}	36.06 ^b	21.61	120.73 ^b	25.69 ^b
		Sd	5.45	66.69	15.37	2.72	16.10	5.81	3.06	13.56	2.85
E-I	Male	\bar{X}	75.21 ^a	623.18 ^{abc}	105.43 ^a	18.22	99.81 ^a	47.16 ^a	21.21	138.07 ^a	31.72 ^a
		Sd	15.76	76.15	7.84	2.58	7.08	3.32	2.80	11.90	3.55
	Female	\bar{X}	58.05 ^{bc}	632.11 ^{ab}	88.74 ^b	17.09	86.30 ^c	33.96 ^b	24.89	119.86 ^b	25.13 ^b
		Sd	13.41	112.42	13.99	2.70	8.97	2.64	7.99	11.11	3.18
E-II	Male	\bar{X}	65.68 ^{abc}	643.92 ^{ab}	112.02 ^a	19.15	98.26 ^{ab}	47.91 ^a	23.38	143.54 ^a	31.03 ^a
		Sd	20.88	70.47	12.05	2.68	6.12	3.66	3.20	12.14	3.68
	Female	\bar{X}	61.38 ^{bc}	561.66 ^c	84.91 ^b	17.79	82.73 ^c	34.51 ^b	22.27	114.72 ^b	23.50 ^b
		Sd	12.55	48.56	13.52	2.24	5.77	3.29	3.30	7.48	3.22

Legend: \bar{X} – average; Sd – Standard deviation

Different superscripts in the same column (a–c) indicate significant differences between groups ($P < 0.05$)

The results obtained show that the dietary treatments had no significant effect on the skin, bone and muscle yields of drumsticks, thighs and breast ($P > 0.05$), except for the breast muscle weight of the female chickens, especially between the E-I and E-II groups ($P < 0.05$). There is not much information in the literature about the effect of the protease enzyme on skin, bone and muscle weights in major carcass cuts. The available research findings indicate that protease supplementation, along with the reduction in crude protein content, had no negative effect on the carcass weight and carcass yield (Rada et al. 2013), proportion of carcass traits, and chemical composition of breast and leg meat (drumsticks and thighs). The carcass yield and relative organ weight were not affected by dietary exogenous protease (Lee et al., 2023).

The E-I female chickens had higher muscle weights than the E-II birds ($P < 0.05$). In all three experimental groups, the male chickens had higher skin, muscle and bone weights in breast, drumsticks and thighs than the female chickens, and the differences were not significant only for the skin weight of drumsticks and thighs ($P > 0.05$). This was an expected result, given the greater body weight of the male chickens of the same age than that of the females. The sex of broilers was found to affect the weights and percentages of individual carcass parts and their tissues (England et al., 2023).

The percentages of skin, bone and muscle in major carcass parts (breast, drumsticks and thighs) across dietary treatments and sexes of 49-day-old chickens are provided in Table 3.

Table 3. The percentages of skin, bone and muscle in the breast, drumsticks and thighs of broilers (%)

Treatment		Yield in breast			Yield in drumsticks			Yield in thighs			
Groups	Sex	skin	muscle	bone	skin	muscle	bone	skin	muscle	bone	
C	Male	\bar{X}	8.30 ^{ab}	78.29 ^{ab}	13.40 ^{ab}	11.62 ^{ab}	61.63 ^{ab}	26.74 ^{bc}	12.05 ^{bc}	71.91	16.03 ^{ab}
		Sd	1.91	2.21	1.41	2.88	2.25	2.27	2.16	3.57	2.54
	Female	\bar{X}	7.55 ^b	80.23 ^a	12.21 ^{ab}	12.14 ^{ab}	62.55 ^{ab}	25.30 ^c	12.92 ^{abc}	71.75	15.33 ^{ab}
		Sd	0.90	2.77	2.53	1.17	2.84	2.77	1.90	2.96	1.61

E-I	Male	\bar{X}	9.47 ^a	77.35 ^b	13.18 ^{ab}	11.04 ^b	60.39 ^{bc}	28.57 ^{ab}	11.13 ^c	72.24	16.63 ^a
		Sd	2.26	2.95	1.16	1.55	1.95	1.67	1.49	2.22	1.62
	Female	\bar{X}	7.71 ^{ab}	80.65 ^a	11.64 ^b	12.45 ^{ab}	62.70 ^a	24.85 ^c	14.58 ^a	70.61	14.81 ^b
		Sd	2.56	4.43	2.60	1.86	3.30	2.93	4.25	4.76	1.65
E-II	Male	\bar{X}	7.91 ^{ab}	78.34 ^{ab}	13.75 ^a	11.55 ^b	59.46 ^c	28.99 ^a	11.80 ^{bc}	72.46	15.74 ^{ab}
		Sd	1.97	1.10	1.78	1.08	1.04	1.31	1.36	2.33	2.14
	Female	\bar{X}	8.72 ^{ab}	79.24 ^{ab}	12.03 ^{ab}	13.15 ^a	61.28 ^{abc}	25.56 ^c	13.83 ^{ab}	71.52	14.65 ^b
		Sd	1.91	2.39	2.04	1.28	2.42	1.87	1.34	2.38	1.85

Legend: \bar{X} – average; Sd – Standard deviation

Different superscripts in the same column (a–c) indicate significant differences between the groups ($P < 0.05$)

The 4% or 6% reduction in dietary crude protein level compared to the C diet and protease supplementation did not affect the skin, bone and muscle percentages of breasts, drumsticks and thighs ($P > 0.05$), except for the bone percentage of drumsticks in the males between the groups C and E-2 ($P < 0.05$). Hidalgo et al. (2004) and Rehman et al. (2017) also found that breast meat and thigh meat percentages were not significantly ($P > 0.05$) affected by dietary protease sources. Li et al. (2023) also reported that dietary protease supplementation had no effect on the breast and thigh muscle percentages in the Arbor Acres broiler chickens at 42 days ($P > 0.05$), whereas Xu et al. (2017) determined that diets supplemented with protease increased the breast muscle weight and breast meat yield of broilers.

As for the effects of chicken sex on the percentages of skin, bone and muscle in major carcass parts, the females had a higher yield of muscle in breast and drumsticks, a higher percentage of skin in drumsticks and thighs, and a lower percentage of bone in drumsticks and thighs than the males ($P < 0.05$). Similar to our research, Da Costa et al. (2017) found a higher percentage of breast meat in females than in male chickens, whereas Fernandes et al. (2013) determined that the broiler sex is the most important factor in breast fillet yield and boneless leg or boned leg yield. Bogosavljević-Bošković et al. (2006) reported that, in the same genotype (Cobb 500), the effect of sex on the skin percentage of breast, drumsticks and thighs was not significant ($P > 0.05$), and that female chickens had a higher muscle percentage of drumsticks and thighs than male broilers, while having a smaller bone percentage in breast, drumsticks and thighs ($P < 0.01$ or $P < 0.05$). The broiler sex significantly affected the bone and muscle percentage of breast and drumsticks, and the bone and skin percentage of thighs (Bogosavljević-Bošković et al., 2011).

CONCLUSION

The research results indicate a negligible effect of different levels of the protease enzyme in broiler diets (200 mg/kg Ronozyme ProAct and 4% crude protein reduction compared to the standard diet or 300mg/kg Ronozyme ProAct and 6% crude protein reduction compared to the standard diet) on the yields and percentages of skin, bone and muscle in the breast, drumsticks and thighs of broilers. Therefore, significant differences were observed only in the breast muscle weights of the E-I and E-II female chicken groups and the bone percentages in the drumsticks of the C and E-2 male chicken groups ($P < 0.05$). In contrast, the effect of sex was much more discernible on the carcass quality parameters, as the males had higher muscle, skin and bone weights and bone percentages in drumsticks and thighs than the females, but also a lower muscle percentage in breast and drumsticks and a lower skin yield in drumsticks and thighs ($P < 0.05$).

On balance, major differences were manifested under the effect of sex, whereas the dietary treatments with 3 levels of protease supplementation had a minor effect on the yield and percentage of skin, bone and muscle in breast, drumsticks and thighs.

Acknowledgements: This study was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, No. 451-03-47/2023-01/ 200088 and No. 451-03-47/2023-01/ 200022.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

- Adebiyi A.O. & Olukosi O.A. (2015): Apparent and standardised ileal amino acid digestibility of wheat distillers dried grains with solubles with or without exogenous protease in broilers and turkeys. *British Poultry Science*, 56: 239-246.
- Anozie A.N., Salami O.A., Babatunde E. Damilola, Babatunde E. Odunayo (2018): Comparative evaluation of processes for production of soybean meal for poultry feed in Nigeria. *Cuban Journal of Agricultural Science* 52(2): 193-202.

- Bogosavljević-Bošković S., Kurčić V., Petrović D.M., Radović V. (2006): The effect of sex and rearing system on carcass composition and cut yields of broiler chickens. *Czech Journal of Animal Science*, 51(1): 31-38.
- Bogosavljević-Bošković S., Pavlovski Zlatica, Petrović D.M., Dosković V., Rakonjac S. (2011): The effect of rearing system and length of fattening period on selected parameters of broiler carcass quality. *Archiv für Geflügelkunde*, 75(3): 158-163.
- Da Costa M.J., Colson G., Frost T.J., Halley J., Pesti G.M. (2017): Straight-run vs. sex separate rearing for two broiler genetic lines Part 2: economic analysis and processing advantages. *Poultry Science*, 96: 2127-2136.
- Dosković V., Bogosavljević-Bošković S., Škrbić Z., Lukić M., Rakonjac S., Petričević V. (2020): Effect of protease added in food on chicken carcass quality. Book of Proceedings XI International Scientific Agriculture Symposium "Agrosym 2020", Jahorina, Faculty of Agriculture, East Sarajevo, Bosnia and Herzegovina, 8-9 October 2020, 829-834.
- Dosković V., Bogosavljević-Bošković S., Škrbić Z., Milošević B., Lukić M., Rakonjac S., Petričević V. (2022): Effects of protease, duration of fattening period and sex of broilers on carcass conformation measures. Book of Proceedings [Electronic source]/XIII International Scientific Agriculture Symposium "Agrosym 2022", Jahorina, Faculty of Agriculture East Sarajevo, Bosnia and Herzegovina, 06-09 October 2022, 1051-1056.
- Dosković V., Bogosavljević-Bošković S., Škrbić Z., Milošević B., Lukić M., Rakonjac S., Petričević V. (2023): Effect of protease added in food and sex on chicken meat classes. Proceedings of the 1st International Symposium on Biotechnology (28th Symposium on Biotechnology with international participation), Faculty of Agronomy, Čačak, University of Kragujevac, Serbia, 17-18 March 2023, 223-229.
- England A., Gharib-Naseri K., Kheravii K.S., Wu Shu-Biao (2023): Influence of sex and rearing method on performance and flock uniformity in broilers – implications for research settings. *Animal Nutrition*, 12: 276-283.
- Fernandes J.I.M., Bortoluzzi C., Triques G.E., Garcez A.F.N., Peiter Daniela Cristina (2013): Effect of strain, sex and age on carcass parameters of broilers. *Acta Scientiarum Animal Sciences*, 35(1): 99-105.
- Hidalgo M.A., Dozier W.A., Davis A.J., Gordon R.W. (2004): Live performance and meat yield responses to progressive concentrations of dietary energy maintained at a constant metabolizable energy-to-crude protein ratio. *Journal of Applied Poultry Research* 13: 319-327.
- Jabbar A., Tahir M., Khan R.U., Ahmad N. (2021): Interactive effect of exogenous protease enzyme and dietary crude protein levels on growth and digestibility indices in broiler chickens during the starter phase. *Tropical Animal Health and Production* 53, 23. <https://doi.org/10.1007/s11250-020-02466-5>
- Lee J., Oh H., Kim Y., Song D., An J., Chang S., Go Y., Cho H., Lee B., Kim W.K., Cho J. (2023): Effects of exogenous protease on performance, economic evaluation, nutrient digestibility, fecal score, intestinal morphology, blood profile, carcass trait, and meat quality in broilers fed normal diets and diets considered with matrix value. *Poultry Science*, 102(5): 102565. doi: 10.1016/j.psj.2023.102565.
- Li X.X., Wang X.X., Lv Y., Ma W.F., Wu X.H., Zhen W.R., Zhao F.R. (2023): Effects of dietary protease supplementation on behaviour, slaughter performance, meat quality and immune organ indices of broilers. *Czech Journal of Animal Science*, 68: 255-265.
- Matkawala F., Nighojkar S., Kumar A., Nighojkar A. (2021): Microbial alkaline serine proteases: Production, properties and applications. *World Journal of Microbiology and Biotechnology*, 37(4): 63. doi: 10.1007/s11274-021-03036-z.
- Park J.H., Lee S.I., Kim I.H. (2020): The effect of protease on growth performance, nutrient digestibility, and expression of growth-related genes and amino acid transporters in broilers. *Journal of Animal Science and Technology* Sep 62(5): 614-627.
- Rada V., Foltyn M., Lichovnicková M., Musilová A. (2013): Effects of protease supplementation of low protein broiler diets on growth parameters and carcass characteristic. *Mendelnet*, 268-272.
- Rehman Z.U., Kamran J., Abd El-Hack M.E., Alagawany M., Bhatti S.A., Ahmad G., Saleem A., Ullah Z., Yameen R.M.K., Ding C. (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.
- Xu X., Wang H.L., Pan L., Ma X.K., Tian Q.Y., Xu Y.T., Long S.F., Zhang Z.H., Piao X.S. (2017): Effects of coated proteases on the performance, nutrient retention, gut morphology and carcass traits of broilers fed corn or sorghum based diets supplemented with soybean meal. *Animal Feed Science and Technology*, 223: 119-127.