

Brazilian Journal of Animal Science e-ISSN 1806-9290 www.rbz.org.br

*Corresponding author:

tiago.petrolli@unoesc.edu.br Received: November 11, 2022 Accepted: August 17, 2023

How to cite: Zanotto, M. J.; Pagnussatt, H.; Valentini, F. D. A.; Dal Santo, A.; Leite, F.; Mis, G.; Zaccaron, G.; Galli, G. M.; Calderano, A. A.; Tavernari, F. C.; Da Silva, A. S.; Paiano, D. and Petrolli, T. G. 2023. Addition of capsaicin in the diet of turkeys: Effects on growth performance and antioxidant and oxidant status in serum and in meat. Revista Brasileira de Zootecnia 52:e20220145.

https://doi.org/10.37496/rbz5220220145

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Non-ruminants Short communication

Addition of capsaicin in the diet of turkeys: Effects on growth performance and antioxidant and oxidant status in serum and in meat

Marlon José Zanotto¹ (D), Heloísa Pagnussatt² (D), Fernanda Danieli Antoniazzi Valentini² (D), Alicia Dal Santo² (D), Felipe Leite² (D), Gilso Mis² (D), Gustavo Zaccaron² (D), Gabriela Miotto Galli³ (D), Arele Arlindo Calderano⁴ (D), Fernando de Castro Tavernari⁵ (D), Aleksandro Schafer Da Silva¹ (D), Diovani Paiano¹ (D), Tiago Goulart Petrolli^{2*} (D)

¹ Universidade do Estado de Santa Catarina, Departamento de Zootecnia, Chapecó, SC, Brasil.

² Universidade do Oeste de Santa Catarina, Xanxerê, SC, Brasil.

- ³ Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Zootecnia, Porto Alegre, RS, Brasil.
- ⁴ Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG, Brasil.
- ⁵ Empresa Brasileira de Pesquisa Agropecuária, Concórdia, SC, Brasil.

ABSTRACT - The objective of this study was to evaluate whether the addition of different levels of capsaicin in the diet of female turkeys has beneficial effects on growth performance and the antioxidant and oxidant status in serum and meat. A total of 150 female turkeys were distributed in a completely randomized design with three treatments with five replicates per treatment and ten birds per experimental unit. Treatments were identified as control (without additive); T400 - basal diet containing 400 mg/kg of pepper extract per kg of feed; and T800 - basal diet supplemented with 800 mg/kg of pepper extract per kg of feed. Growth performance was measured on days 1 and 20 of the experiment. Blood samples were collected at 20 days of the experiment for analysis of the oxidant and antioxidant status, and at 20 days, five birds were euthanized per treatment for the analysis of the oxidant and antioxidant status of the meat. The turkeys fed diet supplemented with capsaicin had lower feed intake and better feed:gain ratio. Turkeys in the T800 treatment showed a reduction in serum levels of reactive oxygen species (ROS) and thiobarbituric acid, and this same effect was observed for ROS in their breast meat. Capsaicin supplementation improves feed conversion and reduces feed intake without altering weight gain in female turkeys. Furthermore, the addition of 800 g/ton reduces lipid peroxidation and protein oxidation in the serum and reduces protein oxidation in broiler turkey meat.

Keywords: antioxidant capacity, capsaicin extract, meat quality, poultry

1. Introduction

Use of antibiotic growth promoters has been widely used to improve growth performance and health status in industrial poultry farming. However, there are doubts about the negative effects of these compounds on human health and the environment (Gonzalez Ronquillo and Angeles Hernandez, 2017), mainly because of the possibility of cross-resistance of pathogenic bacterial strains between humans and animals. Thus, in this scenario, there have been restrictions on the use of antibiotics as growth promoters in animal feed. The European Union banned the use of antimicrobials in 2006 (European Commission, 2005); in addition, countries such as Canada,

Mexico, Japan, and India have limited the use of antimicrobials as growth promoters in animal feed (Salim et al., 2018); moreover, the United States and China have banned their use (Liu et al., 2021). In Brazil, there is also a partial ban on some drugs (Ordinance No. 171/2018; No. 01/2020; MAPA, 2018, 2020).

In this way, companies seek to develop alternative products to replace growth enhancers. Within the proposal of new additives, herbal compounds from plants with functional properties can be an alternative. In this context, capsaicin can be mentioned, as it has anti-inflammatory, antimicrobial, antioxidant, and immunological properties that can result in improved growth performance (Liu et al., 2021). Capsaicin is a compound from chili peppers and is applied in traditional medicine (Hernández-Pérez et al., 2020). Peppers have health-promoting chemical compounds such as capsaicinoids, carotenoids (provitamin A), flavonoids, vitamins (C and E), minerals, and essential oils, which have anti-inflammatory, antioxidant, and antimicrobial properties (Hernández-Pérez et al., 2020). In addition, they provide protection for the microvilli and improve nutrient absorption (Jamroz et al., 2006).

In broiler chickens, the use of capsaicin together with other phytogenics has antioxidant effects (Jamroz et al., 2005), also affecting consumption regulation and metabolic modulation (Pirgozliev et al., 2019). Our hypothesis is that the benefits from phytogenic blends in broilers will be repeated in turkeys with the use of capsaicin as an additive. Therefore, the objective of this study was to evaluate whether the addition of different levels of capsaicin in the diet of female turkeys has beneficial effects on growth performance and the antioxidant and oxidant status in serum and meat.

2. Material and Methods

2.1. Animals, management, and experimental design

The experimental protocol was approved by the Institutional Ethics Committee on the Use of Animals (CEUA) under protocol number 51/2021.

The experiment was carried out in Xanxerê, Santa Catarina, Brazil (Latitude: -26.8364531149; Longitude: -52.4079407059). We used 150 female broiler turkeys, of British United Turkey (BUT) strain, initially weighting 1740±80 g, 45 to 65 days of age, distributed on the first day of housing in a completely randomized design consisting of three treatments and five replicates/ boxes with ten turkeys per treatment, and the pen was considered as the experimental unit for the performance variables.

The turkeys were housed in boxes with a concrete floor, with an area of 1.5 m², set with a tube feeder and a nipple drinker, with wood bedding shavings (depth between 5 and 6 cm), and the lighting program and the management of the environment (temperature, humidity, and ventilation) were kept according to the standard of commercial managements and the breed manual (Aviagen Turkeys, 2015; Table 1). The experimental period was 20 days when the birds received water and feed *ad libitum*. The feed was formulated according to the recommendations of the breed manual. Thus, all groups received the same basal diet, and the only difference was the on-top addition of the additive. The treatments were a basal diet or basal diet with the addition of 2 or 4 mg of capsaicinoids/kg of diet. The source of capsaicinoids used was Capcin[®] (ID4Feed, France), which presented 5 g of capsaicinoids/kg. Then, 400 and 800 mg of the product per kg of the basal diet were added to the feed.

2.2. Performance

Birds were weighed on days 1 and 20 of the experiment. Feed intake (FI) (g/bird/day) was obtained through the difference between the feed provided at the beginning and the leftovers weighed at the end of the period. Feed:gain ratio (FC) was calculated by the total amount of feed ingested divided by the live weight of the birds.

Ingredient	g/kg
Ground corn	474.9
Soybean meal	372.7
Gluten meal	56.0
Bicalcium phosphate	23.5
Limestone	21.0
Soybean oil	23.5
Basemix ¹	28.5
Calculated composition (g/kg as feed basis)	
Metabolizable energy (Mcal/kg)	2.96
Crude protein	256.0
Digestible lysine	15.4
Digestible methionine + cysteine	7.1
Digestible threonine	8.5
Calcium	15.1
Available phosphorus	5.5
Sodium	1.5

Table 1 - Ingredients and basal feed used for all experimental groups

Basemix composition as feed basis: lisine, 140.5 g/kg; methionine, 126.8 g/kg; threonine, 1,260.11 mg/kg; valine, 1,118.82 mg/kg; arginine, 1,496.26 mg/kg; iron, 50.16 mg/kg; zinc, 100.10 mg/kg; manganese, 114.97 mg/kg; cooper, 125.13 mg/kg; iodine, 2.01 mg/kg; selenium, 0.40 mg/kg; vitamin A, 24,802.26 IU/kg; vitamin D, 16,074.43 IU/kg; vitamin E, 77.55 IU/kg; vitamin K3, 10.00 mg/kg; vitamin B1, 7.91 mg/kg; vitamin B2, 24.94 mg/kg; vitamin B6, 15.11 mg/kg; vitamin B12, 58.11 mcg/kg; folic acid, 5.11 mg/kg; biotin, 0.51 mg/kg; niacin, 7.10 mg/kg; pantonenic acid, 44.82 mg/kg; lasalocid, 10.91 g/kg; xylanase, 3.86 g/kg; phytase, 1.76 g/kg; antioxidant, 13.0 g/kg; propionic acid, 11.6 g/kg; citric acid, 20.94 mg/kg; fumaric acid, 47.87 mg/kg; malic acid, 14.96 mg/kg; sorbic acid, 14.96 mg/kg; sodium bicarbonate, 1484.3 mg/kg; *Bifidobacterium animalis*, 19,910,000.00 ufc/kg; *Pediococcus acidilactici*, 49,775,000.00 ufc/kg.

2.3. Sample collection

Blood was collected from five birds per treatment on the 20th day of the experiment. The birds were manually restrained, and blood was collected from the brachial vein using an insulin syringe. Subsequently, this material was placed in a tube without anticoagulant to obtain the serum. After that, this material was centrifuged at 3500 rpm for 10 min, and the serum was separated, collected, and frozen (-20 °C) for the analysis of the oxidant and antioxidant status. Furthermore, at 20 days, five birds per treatment were slaughtered, by cervical dislocation, according to the animal welfare and euthanasia standards described by the CONCEA euthanasia practice guidelines (Brasil, 2013). Fragments of the *pectoralis major* muscle were collected from these animals, and these samples were kept in refrigerated boxes until arrival at the laboratory. Subsequently, a fragment of the muscle was homogenized (saline solution) and centrifuged (2800 g for 10 min), and the supernatant was collected and frozen (-20 °C) until the analysis of pro-oxidants and antioxidants in the meat.

2.4. Oxidant/antioxidant status in serum and meat

Serum and muscle samples were analyzed for reactive oxygen species (ROS) according to the methodology described by Halliwell and Gutteridge (2005), expressed in U DCFH/mg of protein. The thiobarbituric acid reactive substances (TBARS) were analyzed as described by Jentzsch et al. (1996) for serum and by Ohkawa et al. (1979) for meat, which is expressed in nmol MDA/mg of protein. Analyses were also performed for the enzyme glutathione S-transferase (GST; mmol CDNB/min/mg of protein) according to Habig et al. (1974), thiols (mmol SH/mg of protein) according to Ellman (1959), and nitric oxide (NOx; µmol Nox/mg of protein) according to Miranda et al. (2001).

2.5. Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and the Shapiro-Wilk normality test (P>0.05) and then to the Tukey test. All analyses were performed at a 5% significance level, using the R statistical program. The statistical model was as follows:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij},$$

in which Y_{ij} = dependent variable, μ = variable mean, β_i = fixed effect of broilers of the group, and ϵ_{ij} = experimental error associated with observation Y_{ij} .

3. Results

3.1. Performance

The weight gain of the turkeys was not influenced by the treatments (P>0.05). On the other hand, turkeys from treatments with concentrations of 400 and 800 g/ton of capsaicin per kg of feed showed lower feed intake (P = 0.043) and better feed conversion (P = 0.001) when compared with turkeys fed the control diet (Table 2).

Table 2 - Effects of the inclusion of capsaicin on the performance parameters of turkeys from 45 to 65 days of age

Item	Control	400 g/ton	800 g/ton	P-value	CV (%)
Initial body weight (g)	1426	1378	1412	0.212	4.56
Final body weight (g)	2534	2504	2488	0.802	3.90
Weight gain (g)	1385	1407	1392	0.123	7.68
Feed intake (g)	2477a	2312b	2305b	0.043	12.97
Feed:gain ratio	1.79b	1.64a	1.65a	0.001	11.64

a-b - Lowercase letters were used to indicate statistical difference at P≤0.05 level.

3.2. Oxidant/antioxidant status in serum and meat

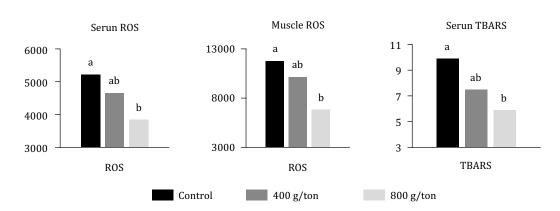
A reduction in serum ROS levels was observed in birds fed diet supplemented with 800 g/ton of capsaicin when compared with those in the control treatment (P = 0.049), and this same effect was verified for serum TBARS levels (P = 0.006; Table 3 and Figure 1). There was a reduction in serum GST levels in birds fed diet supplemented with 800 g/ton compared with those in the treatment with 400 g/ton (P = 0.034; Table 3 and Figure 1). There was a decrease in ROS levels in the meat of birds fed diet supplemented with 800 g/ton of capsaicin in relation to the control treatment (P = 0.011; Table 4 and Figure 1). There was no difference between treatments for thiols and NOx in both serum and meat (P > 0.05; Tables 3 and 4); TBARS and GST variables also had no difference in meat (P > 0.05; Table 4).

Table 3 - Serum antioxidant and oxidant status of turke	ys fed diets supplemented with different levels of capsaicin
Table 5 - Set unit antioxidant and oxidant status of turke	ys led diets supplemented with different levels of capsaicht

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Item	Control	400 g/ton	800 g/ton	P-value	CV (%)
ROS	5214.4a	4652.3ab	3840.9b	0.049	8.54
TBARS	9.90a	7.49ab	5.88b	0.006	10.14
GST	24.27ab	26.55a	21.73b	0.034	7.45
Thiols	0.24	0.24	0.21	0.902	23.45
NOx	0.37	0.68	0.64	0.646	25.65

ROS - reactive oxygen species (U DCFH/mg protein); TBARS - thiobarbituric acid reactive substances (µmol MDA/mL or ŋmol MDA/mg protein); GST - glutathione S-transferase (mmol CDNB/min/mg protein); thiols (mmol SH/mg protein or µmol SH/mL); NOx - nitric oxide (µmol NOx/mg protein); CV - coefficient of variation.

a-b - Lowercase letters were used to indicate statistical difference at P≤0.05 level.



ROS - reactive oxygen species (U DCFH/mg protein); TBARS - thiobarbituric acid reactive substances (µmol MDA/mL or ηmol MDA/mg protein). a-b - Lowercase letters were used to indicate statistical difference at P<0.05 level.

Figure 1 - Effect of different levels of capsaicin on serum and muscle variables in turkeys.

Item	Control	400 g/ton	800 g/ton	P-value	CV (%)
ROS	11784.4a	10151.9ab	6823.8b	0.011	14.32
TBARS	27.22	22.86	28.51	0.346	13.54
GST	46.82	34.04	39.03	0.306	12.08
Thiols	0.39	0.31	0.34	0.602	11.05
NOx	0.77	1.00	1.12	0.785	19.23

Table 4 - Antioxidant and oxidant status in the breast meat of turkeys supplemented with different levels of capsaicin

ROS - reactive oxygen species (U DCFH/mg protein); TBARS - thiobarbituric acid reactive substances (µmol MDA/mL or ŋmol MDA/mg protein); GST - glutathione S-transferase (mmol CDNB/min/mg protein); thiols (mmol SH/mg protein or µmol SH/mL); NOx - nitric oxide (µmol NOx/mg protein); CV - coefficient of variation.

a-b - Lowercase letters were used to indicate statistical difference at P≤0.05 level.

4. Discussion

The lower feed intake obtained in this study with the use of capsaicin was not predicted. Shahverdi et al. (2013) observed an increase in feed intake when the feed for broiler chickens was supplemented with 200 g/ton of ground chili pepper. However, this result was positive, as there was no difference in the weight gain of the turkeys and there was an improvement in feed conversion. Reinbach et al. (2009) showed that the combined use of capsaicin and green tea in humans increases satiety. Thus, the sensitivity of the species studied or the doses used may have promoted a reduction in voluntary intake.

Jamroz et al. (2005) found that the inclusion (100 mg/kg of feed) of a blend of herbal extracts (carvacrol 49.5 g/kg, cinnamaldehyde 29.7 g/kg, and capsaicin 19.8 g/kg) in diets for broiler chickens improved nutrient absorption and intestinal health with reduction in the *E. coli* count and an increase in the *Lactobacillus* spp. concentration, which resulted in an improvement of approximately 4.2% in the feed conversion index. Furthermore, the authors found greater mucus secretion with the creation of a thick layer of mucus in the proventriculus and jejunum wall of birds fed the extracts, and they associated these characteristics with less adhesion of pathogenic bacteria such as *E. coli*, which may explain the better performance of the birds.

Liu et al. (2021), when supplementing 80 mg/kg of capsaicin in the diet of broiler chickens, verified an improvement in the digestibility coefficient of nitrogen-corrected apparent metabolizable energy, digestibility of organic matter, and digestibility of crude protein, which can be explained by the increase in the activity of lipase enzymes and pancreatic trypsin that are related to an improvement in food digestion. The authors also verified an increase in the daily weight gain of birds fed diets supplemented with capsaicin. Thus, the effects on the metabolism previously mentioned for broiler chickens probably occurred in the turkeys of this study given the better feed conversion obtained.

Capsaicin has antimicrobial, analgesic (Vicente et al., 2007), antioxidant, and anti-inflammatory properties (Nascimento et al., 2013; Liu et al., 2021). However, research evaluating the effects of capsaicin in turkeys is scarce. In this study, we found that it could reduce serum TBARS and ROS as well as ROS in broiler turkey meat. The performance of birds can be impaired by oxidative stress that occurs when there is an exacerbated production of free radicals such as ROS, among others. Thus, high ROS levels can induce the production of pro-inflammatory cytokines (Soares et al., 2015), which causes the bird to divert energy to the production of ROS can damage intestinal membranes and thus impair nutrient absorption (Sklyarov et al., 2011). The TBARS is an equivalent of malondialdehyde, which is produced as a byproduct of lipid peroxidation (Jia et al., 2022). In this scenario, 4-hydroxynonenal is the final product of lipid peroxidation, which damages membranes and impairs cell signaling and mitochondrial functions (Mishra and Jha, 2019). Therefore, an increase in TBARS levels is an indicator of an increase in oxidative stress, and this contributes to the lower productive performance of birds. Thus, the improvement in the feed conversion index may have occurred possibly because of the antioxidant effect of capsaicin.

The body has an antioxidant defense system to neutralize excess free radicals composed of enzymatic and non-enzymatic enzymes. Glutathione S-transferase is an antioxidant enzyme present in the cytoplasm of cells, and its main function is to help in the degradation of toxic substances (Kherouf et al., 2021). It breaks down hydrogen peroxide and hydroperoxides into non-toxic products through reduced glutathione. Thus, the lower activity of this enzyme in the treatment with 800 g/ton of capsaicin is due to the lower production of ROS and TBARS, that is, it did not need to increase its activity to combat excess free radicals.

The protein oxidation of meat can be caused by ROS. Thus, ROS-generating systems, such as the lipid-oxidant system, myoglobin-mediated oxidation system, and the metal-catalytic oxidation system (Wang et al., 2019), are important generators of ROS and gain prominence as the excessive production of free radicals can accelerate the deterioration of meat and reduce its shelf life. In this context, the iron atoms present in myoglobin are released by protein degradation and can increase the capacity to produce the hydroxyl radical through the Fenton reaction (Wang et al., 2022). Thus, the result of this reaction is hydroxyl, which can further promote lipid and protein oxidation in meat as a precursor of cascade reactions (Wang et al., 2022). Therefore, it can be assumed that the hydroxyls present in the capsaicin molecule were donated to free radicals and this decreased the production of ROS in the meat, which could increase the shelf life of the meat.

İpçak and Alçiçek (2018) found that supplementation of 150 g/ton of capsicum oleoresin for female broiler chickens improved meat quality through lower thawing loss, lower cooking loss, and increased water holding capacity in thigh meat compared with the control diet. Probably, these positive effects can be explained by the antioxidant activity promoted by capsaicin. Puvača et al. (2016) observed that *Piper nigrum* and *Capsicum annuum* supplementation for broiler chickens reduced lipid peroxidation in the liver and meat because of their antioxidant properties.

Facchi et al. (2023) observed that supplementation of 100 and 150 mg/mg microencapsulated carvacrol and cinnamaldehyde in diets of broilers at 20 days resulted in lower serum levels of TBARS and ROS. Karadas et al. (2014) found that supplementation with a blend composed of 5% carvacrol, 3% cinnamaldehyde, and 2% capsicum oleoresin increased the retention of carotenoids in the liver and coenzyme Q10, which have antioxidant activity. This fact may have occurred in our study, in which carotenoids and coenzyme Q10 donate their methyl groups to free radicals and, consequently, reduce ROS. Kreuz et al. (2022) observed that the supplementation of 1 and 2 mg/kg capsaicinoid has greater mRNA expression superoxide dismutase compared with the control in the jejunum of broilers. This enzyme is responsible to remove the excess of the ROS in the body, which can explain the lower ROS levels in the group that received the supplementation of 800 g/ton.

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5. Conclusions

Supplementation with capsaicin in the diet of turkeys from 45 to 65 days improves feed conversion and reduces feed intake, but it does not change weight gain. The addition of 800 g/ton reduces lipid peroxidation and protein oxidation in the serum, in addition to reducing protein oxidation of the meat.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.J. Zanotto, F.C. Tavernari, A.S. Da Silva, D. Paiano and T.G. Petrolli. Formal analysis: H. Pagnussatt, F.D.A. Vantentini, A. Dal Santo, F. Leite, G. Mis, G. Zaccaron and A.A. Calderano. Investigation: M.J. Zanotto. Methodology: H. Pagnussatt, F.D.A. Vantentini, A. Dal Santo, F. Leite, G. Mis, G. Zaccaron and A.A. Calderano. Project administration: M.J. Zanotto, A.A. Calderano, F.C. Tavernari, A.S. Da Silva, D. Paiano and T.G. Petrolli. Supervision: F.C. Tavernari, A.S. Da Silva, D. Paiano and T.G. Petrolli. Supervision: F.C. Tavernari, A.S. Da Silva, D. Paiano and T.G. Petrolli. Writing – original draft: M.J. Zanotto, G.M. Galli and T.G. Petrolli. Writing – review & editing: M.J. Zanotto, G.M. Galli and T.G. Petrolli.

Acknowledgments

We would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Financial Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Brazil), Universidade do Oeste de Santa Catarina (UNOESC; Santa Catarina, Brazil), and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC; Santa Catarina, Brazil) for the technical support and public funding of research in the country. We also thank the company Nutriquest Brasil for the technical support.

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