Effects of Coccidiosis on the Welfare, Growth Performance, Carcass and Meat Quality of Broilers

Nikola Čobanović[1*], Sara Čalović[2], Nevena Grković[1], Slobodan Knežević[3], Marko Pajić[3]

[1]*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*
[2]*Department of Animal Breeding, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobodjenja 18, 11000 Belgrade, Serbia*
[3]*Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia*

Abstract
Avian coccidiosis compromises welfare and production performance, increases susceptibility to bacterial infections and leads to substantial economic and production losses upon the poultry industry. The objective of this study was to determine the impact of a mixed coccidiosis (*Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella* and *Eimeria necatrix*) challenge on oxidative stress biomarkers, performance indices, carcass and meat quality of broilers. Broilers challenged with coccidiosis had higher concentrations of catalase, glutathione-S-transferase and malondialdehyde and lower concentration of superoxide dismutases at all sampling days. The same group of broilers had a lower feed intake on day 21 and 35, and a lower daily weight gain and body weight on day 35. Healthy broilers had higher hot and cold carcass weights and breast weight, but a lower back and pelvis weight. Also, healthy broilers produced better meat quality in terms of higher water-holding capacity (lower drip and thawing loss in breast and thigh muscle) and less yellow colour (lower b* value in breast and thigh muscle). Chemical composition of healthy broiler meat was better in terms of higher content of dry matter, ash and protein in breast muscle. In conclusion, the presence of coccidiosis in broilers resulted in impaired welfare, poor performance indices and lower carcass and meat quality.

Keywords: broiler coccidiosis, carcass quality, meat quality, oxidative stress, performance indices.

1. Introduction

Avian coccidiosis, an infection caused by protozoa *Eimeria* spp., compromises broiler welfare and production performance, increases susceptibility to bacterial infections and leads to substantial economic and production losses upon the poultry industry all over the world [1–3]. It is estimated that annual global economic losses due to occurrence of coccidiosis in poultry are more than $12.4 billion [4]. The main consequences of *Eimeria* spp. infections are malnutrition, emaciation, skin pigmentation loss, bleeding, intestinal tissue damage, metabolic disturbances, as well as high morbidity and mortality [1–3,5,6]. Compared to healthy broilers, individuals with coccidiosis often show decreased body weight, carcass weight and meatiness, as well as lower mass of primary carcass cuts, such as breast and legs [3,6,7]. Despite the fact that poultry meat is the major source of animal proteins for human nutrition worldwide, limited researches [3,6,8–10] have been conducted on the influence of coccidiosis on meat quality traits in broilers and the results are inconsistent. Several studies reported that the presence of *Eimeria* spp. infection was associated with increased drip and cooking loss, lower protein content and reduced

* Corresponding author: Nikola Čobanović, +381695419491, cobanovic.nikola@vet.bg.ac.rs

redness [3,6,11]. Other researchers [9] reported that the presence of coccidiosis was related to reduced drip loss and lightness, while some investigators [10] found increased lightness in *Eimeria* spp. challenged broilers.

Therefore, the objective of this study was to determine the impact of a mixed coccidiosis (*Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella* and *Eimeria necatrix*) challenge on oxidative stress biomarkers, performance indices and carcass and meat quality of broilers.

2. Materials and methods

A total of 100 Ross 308 male broilers were purchased from a local commercial hatchery and transported to the Scientific Veterinary Institute research facilities, where meticulous sterilization measures were performed using a Blaze disinfection gun to ensure an *Eimeria*-free environment. The experimental procedures were conducted in strict accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes [12] and they were reviewed and approved by the Ethics Committee for Protection and Welfare of Experimental Animals at University of Novi Sad, Serbia (04-81/115). All broilers were housed in 10 rectangular (1 m^2) pens situated in a thermostatically controlled building. Each pen had wood shavings litter (5 cm depth) and one tube feeder and a bell-drinker offering broilers unlimited access to food and water throughout the experiment. Experimental animals were fed with a commercial diet, formulated to meet National Research Council nutrient recommendations [13]. Broilers were fed starter from day 0 to 15, grower from day 16 to 27, finisher from day 28 to 35. During the entire experimental period, routine husbandry procedures were performed, while the light cycle was 20 h light and 4 h dark.

The broilers were randomly allocated to two experimental groups each comprising five replicate floor pens with 10 birds per pen raised to 35 days. The two experimental groups were (i) non-challenge: healthy broilers fed a basal diet without challenge; (ii) challenge: broilers fed basal diet with coccidiosis challenge. At 15 days of age, all broilers from challenge group were orally gavaged with live oocysts (*Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella* and *Eimeria necatrix*) of a hundredfold dose of a commercial vaccine.

To determine oxidative stress biomarkers, blood samples (*v. subcutanea ulnaris* and *v. brachialis*) from six sacrificed broilers from each group were collected in heparinised tubes (BD Vacutainer®, (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) on day 21, 28 and 35. Immediately upon arrival at the laboratory, the blood samples were centrifuged, and plasma aliquots were separated. The red blood cells were rinsed three times in physiological saline solution and frozen at −80 °C until required for testing. Catalase (CAT) and superoxide dismutases (SOD) levels were analysed in the haemolisates and expressed in units/g of haemoglobin, as described in Aebi [14] and Misra and Fridovich [15], respectively. The concentration of glutathione-S-transferase (GST) (mmol of GSH-CDNB conjugate formed/min/mg of haemoglobin) was determined as outlined in Habig et al. [16], while malondialdehyde (MDA) concentration was analysed spectrophotometrically based on the Girotti`s methodology [17].

Average daily feed intake (ADFI), feed conversion rate (FCR), average daily weight gain (ADWG) and body weight were recorded to determine performance indices of broilers. Body weight was determined individually on day 0, 7, 14, 21, 28 and 35, while ADWG, ADFI and FCR were recorded on day 21, 28, and 35. ADWG was calculated based on the following formula: ADWG = (final weight – initial weight) / total experimental days. ADFI was calculated according to the following equation: ADFI = (feed consumed / total experimental days) / number of broilers. FCR was calculated on the basis of the following formula: FCR = ADFI / ADWG.

At 35 days of age, feed was withdrawn for 12 h but water was provided *ad libitum* in order to empty the gastrointestinal tracts. Afterwards, all remaining broilers from both groups were slaughtered at the experimental unit after exposure to CO_2 for 90 seconds, exsanguinated by cutting the neck blood vessels (*a. carotis communi*s and *v. jugularis*) and, then, eviscerated. Each eviscerated carcass was weighed 45 minutes after slaughter to determine hot carcass weight and reweight 24 hours after chilling to obtain cold carcass weight. Thereafter, each broiler carcass was manually cut using a knife into different commercial cuts [18], which were breast, legs (thighs and drumsticks),

wings and back and pelvis. Then, all commercial cuts were weighed using a digital scale (WPS 600/C, Radwag, Radom, Poland).

Breast and thigh samples were further collected and used for the following meat quality analysis: physicochemical indicators (pH and temperature), instrumental colour, water-holding capacity (drip, thawing and cooking loss) and basic chemical composition (content of dry matter, ash, fat and protein). The pH and temperature of breast and thigh were directly measured 15 minutes and 24 hours *postmortem* on the dorsal side using a portable digital pH meter provided with a penetration electrode (Testo 205, Testo AG, Lenzkirch, Germany). Meat colour parameters (L* – lightness, a* – redness, and b* – yellowness) were determined using a portable colorimeter (NR110, 3NH Technology Co., Ltd, Shenzhen, China) equipped with a 4 mm aperture, 2° viewing angle, and D65 illuminant. The colour values were measured 24 hours after slaughter in nine different areas of the breast and thigh samples. For drip loss measurements, each breast and thigh sample were weighed, individually tied with a rope and placed in a hanging position in glass jars, ensuring that the sample was not in contact with the jar. Meat samples were stored in the refrigerator at 4 °C and reweighed after 48 hours. Drip loss was expressed as a percentage of the initial sample weight. All meat samples after drip loss measurement were packed in a plastic freezer bag and placed in a freezer at a temperature of 18 °C. After 48 hours, the samples were thawed at room temperature for 12–16 hours and reweighed to calculate thawing loss presented as percentage weight loss resulting from thawing. The thawed samples were packed in heat resistant (Ziploc) bag and, then, placed in a continuously boiling water bath until the core temperature reached 72 °C, which was measured using a thermometer with a hand probe (Testo 110, Testo AG, Lenzkirch, Germany). The meat samples were allowed to cool until they reached room temperature and, then, reweighed. Cooking loss was determined by weighing meat samples before and after heat treatment, expressed as percentage. For determination of basic chemical composition of breast and thigh samples, dry matter and ash content were determined as described by Association of Official Analytical Chemistry [19], while protein content was determined following the Kjeldahl method [19]. Lipid content was determined based on the Soxlet method [20].

Statistical analysis of the results was performed using with SPSS software (Version 23.0, IBM Corporation, Armonk, NY, USA) [21]. Broilers were classified in two groups: (i) Non-challenge group (healthy broilers); and (ii) Challenge group (broilers infected with coccidiosis). Student t-test was used to examine the differences in oxidative stress biomarkers, growth performance and carcass and meat quality traits in relation to experimental groups. All results were presented by descriptive statistical parameters (mean value and standard deviation). In all cases, statistical significance was accepted at $P < 0.05$.

3. Results and discussion

Effects of coccidiosis challenge on the oxidative stress biomarkers of broilers are shown in Table 1. Broilers challenged with coccidiosis had higher ($P < 0.0001$) concentrations of CAT, GST, MDA and lower ($P < 0.0001$) concentration of SOD at all sampling days, suggesting impaired antioxidant defence and induction of oxidative stress. The obtained results can be explained by the fact that invasion, migration, and replication of *Eimeria* spp. cause damage in the broiler`s organism, especially in blood vessels, internal organs (gizzard) and intestinal wall [3,6,8,22], which results in activation of oxidative stress [6]. Oxidative stress in poultry occurs during and after stressful events including transportation, exercise, and rough handling, and also plays an important role in several diseases, such as respiratory disorders and gastrointestinal problems (e.g., coccidiosis) [3,6,23–25]. Any kind of stress, including inadequate pre-slaughter treatment and/or disease, causes an imbalance between the antioxidant and oxidant levels in the organism and creates an environment in which cells undergo oxidative stress [6,26,27]. This results in marked alterations in oxidative stress biomarkers [3,24,25,28], which can explain higher CAT, GST activity and MDA concentration, and lower SOD activity in broilers challenged with coccidiosis (Table 1). Contrarily, under normal conditions, i.e. when animals are not sick and/or stressed, they are able to maintain normal body homeostasis and metabolism, thereby avoiding activation of oxidative stress [27].

Effects of coccidiosis challenge on the performance indices of broilers are reported in Table 2. The *Eimeria* challenge in broilers reduced ADFI on day 21 ($P = 0.0500$) and 35 ($P = 0.0489$), as well as ADWG ($P = 0.0500$) and body weight ($P = 0.0311$) on day 35. Previous studies [3,4,6,8] reported substantial negative effects of *Eimeria* spp. infection on growth performance of broilers. The destruction of the intestinal wall, i.e. alterations in gut morphology and truncation of the intestinal villi due to presence of parasites, causes a reduction in feed intake, increase metabolic demands, catabolism of muscle, bone and fat tissue synthesis, impair nutrient transport to target tissue and also interrupts nutrient digestion, absorption and assimilation, generating changes in protein, carbohydrate, lipid, and macro and micro mineral metabolism [1,2,3,5,6,8]. This leads to reprioritisation and repartition of nutrients (amino acids) from the productive processes, including muscle deposition and bone formation, to those processes that require considerable nutritional support, such as synthesis of plasmatic protein, repairing of the damaged gastrointestinal tissue and mucus replacement [29]. All this can explain poor performance indices being found in broilers with coccidiosis (Table 2).

Table 1. Effects of coccidiosis challenge on the oxidative stress biomarkers of broilers (mean ± standard deviation)

Items	Non-challenge	Challenge	*P*-value
Catalase (U/g Hb)			
Day 21	13.20 ± 1.13	27.58 ± 1.47	<0.0001
Day 28	13.48 ± 0.74	31.17 ± 2.04	<0.0001
Day 35	13.06 ± 0.95	33.01 ± 1.81	<0.0001
Superoxide dismutases (U/g Hb)			
Day 21	62.52 ± 1.95	31.95 ± 1.71	<0.0001
Day 28	71.34 ± 1.73	31.27 ± 1.58	<0.0001
Day 35	70.98 ± 1.64	30.44 ± 1.29	<0.0001
Glutathione-S-transferase (U/g Hb)			
Day 21	111.90 ± 4.74	136.83 ± 4.82	<0.0001
Day 28	112.66 ± 2.00	151.46 ± 4.06	<0.0001
Day 35	110.80 ± 2.57	160.55 ± 2.37	<0.0001
Malondialdehyde (U/g Hb)			
Day 21	3.39 ± 0.88	8.16 ± 0.72	<0.0001
Day 28	3.68 ± 1.03	11.09 ± 0.84	<0.0001
Day 35	3.63 ± 0.92	14.08 ± 1.19	<0.0001

Table 2. Effects of coccidiosis challenge on the performance indices of broilers (mean ± standard deviation)

Items	Non-challenge	Challenge	*P*-value
Average daily feed intake (g)			
Day 21	44.19 ± 0.38	41.49 ± 4.79	0.0500
Day 28	57.31 ± 3.54	59.55 ± 6.70	0.7166
Day 35	83.49 ± 4.95.483	71.94 ± 8.16	0.0489
Average daily weight gain (g)			
Day 21	29.91 ± 5.48	28.34 ± 5.32	0.1481
Day 28	37.65 ± 4.93	38.13 ± 4.8	0.7047
Day 35	45.48 ± 7.32	39.00 ± 6.96	0.0500
Feed coversion rate			
Day 21	1.36 ± 0.01	1.36 ± 0.20	>0.9999
Day 28	1.42 ± 0.08	1.50 ± 0.09	0.4858
Day 35	1.70 ± 0.11	1.79 ± 0.11	0.5097
Body weight (g)			
Day 0	40.25 ± 1.48	38.98 ± 1.79	0.0002
Day 7	137.80 ± 17.95	140.40 ± 21.87	0.5173
Day 14	363.30 ± 57.58	358.70 ± 61.19	0.6959
Day 21	668.40 ± 115.10	638.80 ± 113.90	0.1972
Day 28	1094.00 ± 138.00	1112.00 ± 136.30	0.6121
Day 35	1566.00 ± 302.40	1404.00 ± 243.70	0.0311

Table 3. Effects of coccidiosis challenge on the carcass composition of broilers (mean ± standard deviation)

Items	Non-challenge	Challenge	*P*-value
Hot carcass weight (g)	1446.00 ± 134.00	1247.00 ± 162.90	0.0094
Cold carcass weight (g)	1436.00 ± 122.20	1222.00 ± 159.60	0.0053
Breast weight (g)	426.90 ± 65.12	372.90 ± 64.47	0.0500
Leg weight (g)	162.30 ± 31.83	153.40 ± 20.53	0.2949
Wing weight (g)	87.46 ± 7.60	82.23 ± 14.25	0.3007
Back and pelvis weight (g)	234.60 ± 49.07	291.70 ± 59.54	0.0446

Table 4. Effects of coccidiosis challenge on the meat quality of broilers (mean ± standard deviation)

Items	Non-challenge	Challenge	*P*-value
Breast			
pH_{15min}	6.42 ± 0.18	6.39 ± 0.13	0.6695
T_{15min}	32.78 ± 3.75	32.41 ± 2.15	0.7863
pH_{24h}	5.73 ± 0.1	5.78 ± 0.11	0.3166
T_{24h}	2.64 ± 0.95	2.49 ± 0.76	0.7034
Drip loss (%)	3.81 ± 1.56	4.99 ± 1.61	0.0235
Thawing loss (%)	7.26 ± 3.59	8.40 ± 3.65	0.0445
Cooking loss (%)	23.76 ± 5.86	23.72 ± 4.71	0.9863
L* (lightness) value	48.78 ± 2.87	48.36 ± 2.08	0.7079
a* (redness) value	1.85 ± 0.86	1.75 ± 0.63	0.7671
b* (yellowness) value	4.90 ± 1.32	5.54 ± 1.19	0.0359
Thigh			
pH_{15min}	6.38 ± 0.28	6.43 ± 0.17	0.5904
T_{15min}	29.12 ± 4.06	29.49 ± 3.13	0.8181
pH_{24h}	6.10 ± 0.22	6.12 ± 0.20	0.7227
T_{24h}	2.81 ± 0.80	3.03 ± 0.74	0.5194
Drip loss (%)	0.93 ± 0.47	1.81 ± 0.71	0.0031
Thawing loss (%)	3.32 ± 1.59	4.26 ± 1.28	0.0407
Cooking loss (%)	27.88 ± 5.32	27.85 ± 5.34	0.9906
L* (lightness) value	49.09 ± 2.58	48.77 ± 3.03	0.7970
a* (redness) value	2.28 ± 0.70	2.99 ± 0.84	0.4355
b* (yellowness) value	3.81 ± 1.75	4.31 ± 1.06	0.0465

Table 5. Effects of coccidiosis challenge on the chemical composition of broiler meat (mean ± standard deviation)

Items	Non-challenge	Challenge	*P*-value
Breast			
Dry matter (%)	22.10 ± 0.69	20.90 ± 0.77	0.0600
Ash (%)	1.05 ± 0.04	0.98 ± 0.02	0.0315
Fat (%)	0.65 ± 0.19	0.76 ± 0.17	0.3989
Protein (%)	20.41 ± 0.40	19.11 ± 0.56	0.0096
Thigh			
Dry matter (%)	20.00 ± 0.43	19.88 ± 0.64	0.7654
Ash (%)	0.99 ± 0.03	0.94 ± 0.03	0.0930
Fat (%)	1.31 ± 0.28	1.14 ± 0.21	0.3601
Protein (%)	17.80 ± 0.58	18.37 ± 0.38	0.1522

Effects of coccidiosis challenge on the carcass composition of broilers are presented in Table 3. Broilers challenged with coccidiosis had lower hot carcass weight (P = 0.0094), cold carcass weight (P = 0.0053) and breast weight (P = 0.0500), but a higher weight of back and pelvis (P = 0.0446), indicating lower carcass quality in individuals with parasitic disease. As previously mentioned, coccidiosis in broilers and, thus, longer lasted stimulation of the immune system, results in lower feed intake, less efficient conversion of available feed to body mass, decreased daily weight gain and, consequently, uneven, and reduced growth [2,3,5,6,8]. As a consequence of metabolic disorders associated with the presence of coccidiosis, broilers ingest fewer nutrients than what is necessary for the maximum expression of their genetic potential for protein deposition [30],

which leads to decreased meat yield in terms of reduced final body weight, carcass weight and mass of primary carcass parts, such as breast and legs [6,7,31]. All this can explain significant deterioration in carcass quality traits of challenged broilers (Table 3), which is further connected with tremendous economic losses for farmers and the poultry industry as a whole [1–3].

Effects of coccidiosis challenge on the meat quality of broilers are shown in Tables 4 and 5. Broilers challenged with coccidiosis had higher drip loss (P = 0.0235 and P = 0.0031, respectively), thawing loss (P = 0.0445 and P = 0.0407, respectively) and b* value (P = 0.0359 and P = 0.0465, respectively) in breast and thigh muscle. In contrast, meat obtained from healthy broilers had higher content of dry matter (P = 0.0600), ash (P = 0.0315) and protein (P = 0.0096) in breast muscle, indicating higher nutritional value of meat in individuals free from coccidiosis (Table 5). Coccidiosis acts as a stressor stimulus to broiler organism, which causes inflammation, oxidative stress and alters the postmortem metabolism in the skeletal muscles, and, thus, negatively affects meat quality [30]. Several studies [3,6,8–11,32] reported that the presence of *Eimeria* spp. infection in broilers resulted in poor water-holding capacity (increased or reduced drip and cooking loss) and unfavourable colour of meat (reduced redness and reduced or increased lightness). Less red and more yellow meat colour of broilers with coccidiosis can be ascribed to the fact that these intestinal parasites increase blood loss (as a consequence of bleeding in caecum) and decrease content and absorption of minerals (especially iron) in skeletal muscle tissue [3,6,11]. Furthermore, lower protein content in challenged group of broilers can be explained by the fact that coccidiosis decreases the efficiency of digestion, absorption and assimilation of nutrients, which leads to a reduction in the protein synthesis and increases its degradation rates [6]. Considering that water is (firmly or loosely) bound to proteins in meat [33], low protein content can explain higher drip and thawing loss being found in meat from broilers infected with *Eimeria* spp. (Table 4). Also, lipid oxidation induces alterations in protein structures of skeletal muscles in broilers with coccidiosis and, thus, affects the water-holding capacity, leading to higher water loss postmortem [11].

4. Conclusions

This study showed that the presence of coccidiosis in broilers resulted in impaired welfare, poor performance indices and lower carcass and meat quality. Based on the results of this and earlier studies, the association between *Eimeria* spp. infection and meat quality characteristics of broilers is not clear and, therefore, warrants further investigation to determine their potential causal effects. Considering high economic losses in primary production and meat industry, more research is necessary to determine the possible use of different natural anticoccidial substances in broiler production, and their influence on welfare, growth performance and carcass and meat quality.

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