Effects of subclinical Eimeria tenella infection on Pectoralis major muscle in broiler chickens

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ABSTRACT
The aim of this study was to evaluate changes in the histological characteristics of the pectoralis major (PM) muscle in chickens as well as histological changes in the caecum after a low dose of Eimeria tenella (E. tenella). The chickens were inoculated orally with $5 \times 10^2$ E. tenella oocysts (Gr1) and $1 \times 10^3$ E. tenella oocysts (Gr2) at 16 days of age. Six chickens from each group were sacrificed for post mortem examination at 9 days post-infection (pi) and 16 days pi. Chicken growth was not affected by infection. On the 9th day of infection, caecal villus height was significantly greater in the non-infected control group. However, infected chickens from group Gr1 sacrificed seven days later had an even greater caecal villus height than those of the control ($p = .001$). Both infected groups had higher PM weights at 16 days pi than did the control group ($p = .001$). The fibre cross-sectional area was smaller in Gr2 at 9 days pi; however, this parameter was larger in these chickens at 16 days pi. The pH value of PM of group Gr2 was significantly higher than that of the control group or group Gr1 at 16 days pi ($p = .020$).

Introduction
Coccidiosis is one of the most detrimental enteric diseases and is a major economic problem for the poultry industry (Wang et al. 2013). Despite the hidden danger of subclinical coccidiosis in the poultry industry, there appear to be very few studies available about the effects associated with this form of infection. Although broilers are a main source of protein worldwide, there are as yet no studies dealing with the effects of coccidiosis on the histopathology of muscle tissue in these birds.

The objective of this study was to evaluate the histological changes in caecum infected with subclinical doses of E. tenella. The main part of the study, however, determines whether a low dose of E. tenella also affects the structure of the pectoralis major muscle (PM), as the most frequently consumed part of broiler chickens.

Materials and methods
The experiment was conducted in the poultry house of the Czech University of Life Sciences Prague. Thirty-six (one-day-old) broiler chickens (ROSS 308, males) were reared under standard sanitary and environmental conditions. To avoid contamination, control and infected groups were housed in separate cleaned and disinfected rooms under identical conditions. Feed (without anthelmintics or anticoccidial drugs) and water were provided ad libitum. During the experiment, chickens were fed three feed mixtures (starter, grower and finisher diet). On day 1, birds were weighed and individually tagged. The chickens ($n = 36$) were placed in three pens (12 individuals per pen) with one treatment per pen. Birds from Group1 (Gr1) and Group2 (Gr2) were at 16 days of age infected per os with a 1 ml dose containing $5 \times 10^2$ E. tenella oocysts and $1 \times 10^3$ E. tenella oocysts, respectively, directly into the oesophagus using a needleless syringe. Eimeria tenella isolates were obtained from the BIOPHARM Research Institute of Biopharmacy and Veterinary Drugs, Jiřově u Prahy. The chickens were weighed once every 7 days. Six chickens from each group were euthanatised by cervical dislocation at day 9 post-infection (pi) (at 25 days of age). All...
experimental procedures were conducted in accordance with Czech legislation (section 29 of Act No 246/1992 Coll., on the protection of animals against cruelty, as amended by Act No 77/2004 Coll.).

Post mortem, caecal segments were collected, fixed in 10% formalin, dehydrated in absolute alcohol, cleared in xylene and embedded in paraffin blocks; sections were cut at a thickness of 5 μm and subjected to routine haematoxylin and eosin staining. Caecal villus height was estimated by measuring the vertical distance from the villus tip to the villus-crypt junction level for 40 villi per section.

Pectoralis major (PM) samples were collected immediately after slaughter at 9 and 16 days pi in order to determine the histochemical parameters of the muscle fibres. The samples were frozen in 2-methylbutane cooled by liquid nitrogen (−156°C) and then stored at −80°C until analysis. For each sample, the cross-sections (12 μm thickness) were cut at −20°C using a Leica CM 1850 cryostat (Leica Microsystems Nussloch GmbH, Nussloch, Germany). Staining for myofibrillar ATPase after alkaline preincubation, was performed according to Brook and Kaiser (1970). Image analysis (NIS Elements AR 3.1, Nikon, Tokyo, Japan) was used to determine number of muscle fibres per 1 mm² and the fibre cross-sectional area.

The pH value was measured 24 hours post mortem using a Jenway 3510 (Jenway, Essex, England) calibrated pH metre with a glass core probe embedded 1 cm deep in the transversal section of PM. Colour characteristics, according to CIELab scale parameters for L* (lightness), a* (redness) and b* (yellowness), were determined 24 hours post mortem on the cross-section of the PM using a spectrophotometer Minolta SpectraMagic™ NX (Konica Minolta Sensing, Inc., Osaka, Japan). Meat tenderness was determined by the Warner-Bratzler shear test in cooked PM samples (80°C for 1 h). Cooled meat samples were cut into 2 × 1 cm² cuboids, with the cuts running perpendicular to the muscle fibres. An Instron Model 3342 (Instron, Norwood) with a shear blade containing a triangular hole was used to measure meat tenderness. The cooking loss was determined by calculating the difference between the weights of the raw and cooked PM samples.

Results were evaluated using the SAS 9.4 programme (SAS Institute Inc.) ANOVA methods. Growth was assessed by a one-way analysis of variance. Duncan’s multiple range test was used to appraise differences between the groups. PM quality, histology of PM and caecum were evaluated by a two-way analysis of variance. The t-test was used to evaluate differences between values of group and age interactions. All data were expressed as mean ± standard deviation values. Differences of p ≤ .05 between mean values were considered statistically significant.

Results

Differences in live weight were not statistically significant (Table 1). No birds became severely ill or died during the experiment. On the 9th day of infection, villus height was significantly greater in the non-infected control group and decreased (p < .001) with infection intensity. However, infected chickens (Gr1) sacrificed 7 days later had an even greater villus height than those of the control (p < .001).

PM weight was not affected 9 days pi with E. tenella. However, at the time of the second slaughter when the animals reached 32 days of age (16 days pi), both infected groups had higher PM weights than the control group (p < .001).

Muscle fibre numbers were not affected by coccidiosis and decreased with age (p < .006). The cross-sectional area of the control chickens GrC was similar to that of the group with low-grade infection (Gr1) on day 9 and 16 pi (p < .001). However, when compared with the control group, the cross-sectional area of group Gr2 was significantly smaller 9 days pi (25 days of age) and larger 16 days pi (32 days of age). The cross-sectional area increased with age (p < .001) in all groups.

The pH value was significantly affected by interaction between the coccidia oocyst dose and bird age (p < .020), although no differences were observed between all groups 9 days pi (Table 1). However, the pH value of group Gr2 was significantly higher than that of the control group or group Gr1 at 16 days pi (32 days of age). The E. tenella infection did not reveal any significant differences with respect to meat colour parameters.

Meat tenderness was significantly affected by coccidial oocysts (p < .032). Group Gr2 had significantly softer meat than group Gr1 16 days pi. However, control chickens did not differ from either of the infected chicken groups.

Discussion

The severity of disease is influenced by multiple factors including parasite and host genotype, poultry management system, previous exposure history and especially oocyst dose (Blake 2015). According to Conway and McKenzie (2007), a dose of 10⁴ to 10⁵ sporulated E. tenella oocysts is required to induce a severe clinical response in broilers.

The destruction of the intestinal wall causes a reduction in feed intake, a higher feed conversion ratio
and a decrease in the ability to absorb sufficient nutrients (Jatau et al. 2014). The decreased weight gain and weight observed in the infected birds may be attributed to changes in gut morphology and truncation of the intestinal villi; this is a result of injury caused by parasite invasion and replication, thereby affecting nutrient absorption (Shabban 2012). In the present study, chickens growth was not affected by infection. The effects of low doses of E. tenella oocysts (3/10^3) were studied by Jatau et al. (2014), who also reported histopathological changes in the intestines. In our study, villus height was influenced by both doses of E. tenella oocysts. Changes in intestinal morphology, such as shorter villi and deeper crypts, which were also detected in restricted rabbits in a study published by Tumová et al. (2016), have been associated with a higher tissue turnover or the presence of toxins (Miles et al. 2006).

PM weight differed between groups at the time of the second slaughter and was higher in inoculated groups than in the control group. Weight gain in the PM was presumably affected by muscle fibre size. The cross-sectional area of muscle fibres was larger in infected chickens than in the control at the same age. The effects of low-grade E. tenella infection seem to mimic feed restriction, which has been used in broiler chickens to improve health conditions as well as certain production parameters (Ozkan et al. 2006). Our results indicate similar trends to those described by Velleman et al. (2014) in restricted chickens. Our experiment noted that PM muscle weight of the infected chickens was significantly higher than that of birds in the control group on day 18 pi. Moreover, the fibre cross-section area was smaller in infected chickens from Gr2 at 9 days pi; however, this parameter was larger in chickens from the same group (Gr2) at 18 days pi. These results indicate that chickens recover from infection and begin to grow rapidly. Higher doses of coccidian oocysts had a greater effect on pH u value at 16 days pi. Potkowicz et al. (2015) detected a similar effect on pHu value at 16 days pi. Potkowicz et al. (2015) detected a similar effect on pHu value at 16 days pi. Potkowicz et al. (2015) detected a similar effect on pHu value at 16 days pi. Potkowicz et al. (2015) detected a similar effect on pHu value at 16 days pi. The PM was presumably affected by muscle fibre size. The PM was comprised of different muscle fibres, including red and white fibres. The effects of low-grade E. tenella infection seem to mimic feed restriction, which has been used in broiler chickens to improve health conditions as well as certain production parameters (Ozkan et al. 2006). Our results indicate similar trends to those described by Velleman et al. (2014) in restricted chickens. Our experiment noted that PM muscle weight of the infected chickens was significantly higher than that of birds in the control group on day 18 pi. Moreover, the fibre cross-section area was smaller in infected chickens from Gr2 at 9 days pi; however, this parameter was larger in chickens from the same group (Gr2) at 18 days pi. These results indicate that chickens recover from infection and begin to grow rapidly. Higher doses of coccidian oocysts had a greater effect on pH u value at 16 days pi. Potkowicz et al. (2015) detected a similar trend in restricted chickens. Breast colour in chickens infected with E. tenella did not differ from that of the control group. E. tenella infection had no effect on cooking loss, and this observation is in accordance with the results of feed restriction studies described by Butzen et al. (2013). Meat tenderness was significantly lower in Gr2, which was inoculated with a higher dose of oocysts. The effects of E. tenella infection did not seem to affect meat quality parameters negatively.

Table 1. Effect of E. tenella on live weight, caecum histology and selected meat quality parameters.

|                       | 9 days pi                   | 16 days pi                   | Significance |
|-----------------------|-----------------------------|-----------------------------|--------------|
| Live weight, g        | GrC 1070 ± 49.5             | Gr1 1173 ± 44.9             | Gr2 1105 ± 95.8 |
| Caecal villus height, μm | 207±95.1                    | 141±43.0                    | 106±31.5     |
| Breast weight, g      | 165 ± 3.7                   | 173 ± 5.0                   | 161 ± 10.2   |
| Number of muscle fibres per 1 mm | 514 ± 98.9                  | 535 ± 104.8                 | 505 ± 77.0   |
| Cross sectional area, μm² | 1630 ± 649.0                | 1543 ± 702.0                | 1279 ± 565.2 |
| pH 24                 | 5.90±0.2                    | 5.75±0.1                    | 5.84±0.2     |
| Colour parameters     |                             |                             |              |
| L*                    | 52.72 ± 3.3                 | 55.22 ± 23                  | 52.74 ± 2.5  |
| a*                   | −1.61 ± 0.8                 | −1.43 ± 0.7                 | −1.91 ± 0.6  |
| b*                   | 7.71 ± 1.8                  | 9.62 ± 1.1                  | 7.83 ± 1.5   |
| Cooking loss, %       | 22.07 ± 3.4                 | 20.55 ± 40                  | 20.04 ± 3.5  |
| Fmax, N               | 30.22 ± 6.5                 | 29.89 ± 8.0                 | 26.02 ± 6.7  |

GrC: control group; Gr1: group infected with 5 × 10⁵ oocysts; Gr2: group infected with 1 × 10⁶ oocysts; L*: lightness; a*: redness; b*: yellowness; Fmax: maximum shear force.

a–eMeans listed in the same row with different superscripts differ significantly (p ≤ 0.05).
Conclusions
In summary, the main objective of this study was to evaluate whether mild E. tenella infection induces PM muscle changes. Subclinical doses of E. tenella caused significant reductions in muscle fibre cross-sectional area and caecal villus height among infected chickens at 9 days pi. However, infected chickens had significantly higher PM muscle weight, the largest muscle fibre cross-sectional area and the greatest caecal villus height at 18 days pi. The effect of low doses of coccidia on meat quality is similar to those generated by feed restrictions implemented by chicken breeders.

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Disclosure statement
The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of this article.

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