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Rapid growth predisposes broilers to necrotic enteritis

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Abstract

Over the past 50 years, intentional genetic selection within the broiler industry has led to major improvements in both body weight gain (BWG) and feed conversion efficiency. Next to its economic advantages, enhancing BWG can increase the risk for metabolic and skeletal disorders. The aim of this study was to examine whether higher BWG is a predisposing factor for broiler necrotic enteritis.

In this study, 300 broilers were challenged with *Clostridium perfringens* using a well-established, previously described challenge model. It was found that birds with a higher body weight (BW) and BWG before challenge were predisposed to develop more severe necrotic enteritis lesions. After challenge, the average BWG of the birds developing mild to severe lesions dropped significantly, negatively affecting animal welfare and performance. These results show a significant interplay between BWG and the development of necrotic enteritis lesions. This raises the question whether there is a limit to broiler performance with respect to maintaining intestinal health, and whether decreasing BWG (at certain stages of the growth cycle) can be part of a plan to prevent intestinal pathology.

Research highlights

Higher body weight is a predisposing factor to necrotic enteritis in broilers.

Introduction

The poultry industry has gained importance within the agricultural world throughout the years. Every year, over 90 million metric tonnes of broiler meat is being produced worldwide and this value is forecasted to grow another 24% within the next decade (USDA, 2018) (Kuberka et al., 2017) (Watt Global Media, 2016). In order to meet the rising demand to reduce overall production cost and to increase profitability, the industry focusses on maximization of economically important parameters such as body weight gain (BWG) and feed conversion efficacy (FCE). Consequently, the slaughter weight is reached earlier and feed cost is reduced. Improvements are made through genetic selection, the use of feed additives and adjusting feed composition (Hunton, 2008) (Huyghebaert et al., 2011). Together, this has led to major changes in both broiler appearance and performance, increasing body weight and muscle yields and decreasing feed conversion rate (Hunton, 2008) (Zuidhof et al., 2014).

Despite its clear economic advantages, rapidly increasing BWG has a high burden on animal health, leading to an increase in metabolic, musculoskeletal and cardiovascular disorders in broilers with the highest productivity (Angel, 2007) (Lilburn, 1994) (Kestin et al., 1992) (Rath et al., 2018) (Williams et al., 2004) (Kumari et al., 2016) (Scheele, 1997) (Peacock et al., 1990) (Olkowski, 2006) (Mazzoni et al., 2015) (Kuttappan et al., 2016). In contrast to the effect of increased BWG on susceptibility to metabolic diseases, the impact on infectious diseases is less well described but plausible. Cheema et al. (2003) describes the alteration of the immune response to pathogens in fast-growing broilers, resulting in a decline in disease resistance.

One pathogen that is tightly linked to animal production under intensive rearing conditions is *C. perfringens* (Songer et al., 2016). Overeating disease is a well-known phenomenon in different species of production animals, in which animals that consume more feed tend to suffer more from *C. perfringens* related illnesses (Lebrun et al., 2010) (Songer, 1996). Necrotic enteritis (NE) in broilers,

caused by NetB+ *C. perfringens* strains, is characterised by multifocal to coalescent necrotic lesions in the small intestine. This results in a reduced growth rate, a poor feed conversion efficacy (subclinical NE) and eventually death (clinical NE). Co-occurrence of *C. perfringens*-associated hepatitis can increase carcass condemnation rates at slaughter (Lövland & Kaldhusdal, 1999). The disease occurs after massive proliferation of *C. perfringens* in a favourable gastrointestinal environment resulting in toxin production (McDevitt et al., 2006). Multiple predisposing factors have been described that can change the physical properties of the gut (by damaging the epithelial surface, changing the gut transit time or mucus production), disrupt the gut microbiota or alter the immune status of the animal (Moore, 2016). Furthermore, management factors (crowding of the birds, low ambient temperature, wet litter), nutritional factors (non-starch polysaccharides, increased feed viscosity and high protein content, especially protein of animal origin) and other infectious agents (coccidiosis) can be of predisposing significance (McDevitt et al., 2006) (Broom, 2017). While rapid growth has been shown to predispose to various metabolic and skeletal disorders in broilers, the predisposing effect of high BWG on this intestinal disease has not yet been investigated. The aim of the current study was to evaluate the interaction between growth rate and NE disease severity, using an established *in vivo* model.

Keywords

Necrotic enteritis; Broiler; Body weight gain; Predisposing factor

Material and Methods

Necrotic enteritis in vivo trial

Three hundred broilers (mixed sex) were housed in the same stable. A well-established NE challenge model was used with minor modifications (Gholamiandehkordi et al., 2007). Figure 1 represents a timeline of the NE *in vivo* protocol. In short, water and feed were supplied ad libitum. The feed was a wheat/rye-based (43%/7.5%) diet containing soybean meal as a protein source. At 17 days of age, the soybean meal was replaced by fishmeal (30%), increasing the amount of animal protein which is a predisposing factor for induction of NE. A mild form of immunosuppression was induced by oral administration of an infectious bursal disease vaccine (Nobilis Gumboro D78 vaccine, Schering-Plough Animal Health, Brussels, Belgium) at days 4 and 9. Mild intestinal damage was induced through oral administration of a tenfold dose of a live coccidiosis vaccine at day 14 and day 16 (day 14: Hipracox, Hipra, Gerona, Spain; day 16: Paracox-5, MSD Schering-Plough Animal Health, Brussels, Belgium). All broilers were challenged with a well-characterized NetB+ *C. perfringens* (CP56, Gholamiandehkordi et al., 2007), resulting in the induction of subclinical NE. Here for, at days 17, 18 and 19, one millilitre overnight culture (in Brain heart infusion broth (Bio-Rad, Temse, Belgium, product number 64014)) of the pathogenic *C. perfringens* strain CP56 was orally administered to each bird. At day 20, birds were euthanized. The experiments were carried out according to the recommendations and following approval from the Ethical Committee of the faculty of Veterinary Medicine at Ghent University (EC2016_127).

Broilers were weighed four days before and one hour before *C. perfringens* challenge (day 13 and 17 respectively) and after the challenging period (day 20). The average daily BWG before and during challenge were calculated as the (body weight (BW) at day 17 – BW at day 13) divided by 4 days and (BW at day 20 – BW at day 17) divided by 3 days, respectively. At necropsy (day 20), the lesions in the duodenum, jejunum and ileum were scored using a well-established scoring system (Gholamiandehkordi et al., 2007). In short, score 0: no gross lesions; score 2: focal necrosis and

ulceration (1-5 foci); score 3: focal necrosis and ulceration (6-15 foci); score 4: focal necrosis and ulceration (16 or more foci); score 5: patches of necrosis 2 to 3 cm long and score 6: diffuse necrosis.

Statistical analysis

In order to gain more insight into the link between BW or average daily BWG and NE disease severity, the data of the NE trial were grouped according to disease severity. The data were divided into 4 groups: No lesions (score 0; n=117), Mild (score 2; n=67), Moderate (score 3 and 4; n=66) and Severe (score 5 and 6; n=50). The results were analysed using Graphpad Prism 8. Normality was checked using the D'Agostino and Pearson omnibus normality test. The non-parametric Kruskall-Wallis test was used to compare the means of BW or average daily BWG between groups (No lesions, Mild, Moderate and Severe). A pairwise comparison was made using a Dunn correction at an overall significance level of 5%. The given p-values were adjusted to the multiple pairwise comparison. Quantitative differences between means of groups are given with their corresponding SEM value, calculated using the baseline correction function of GraphPad Prism. A two-tailed paired t-test was used to compare BWG before and after *C. perfringens* challenge. The correlation coefficient was obtained using the Spearman correction method.

Results

Higher body weight and growth rate before C. perfringens challenge is linked to an increased severity of necrotic enteritis

The effect of the initial BW of the birds before *C. perfringens* challenge on the outcome of necrotic enteritis lesion development was evaluated both at 4 days before (day 13) and one hour before (day 17) *C. perfringens* challenge. When comparing the BW of birds that did not develop NE to birds that developed moderate to severe NE, a significant difference could be observed both at four days before (no lesions vs. moderate NE: p=0.0044; no lesions vs. severe NE: p=0.0072) and at one hour before *C. perfringens* challenge (no lesions vs. moderate NE: p=0.0362; no lesions vs. severe NE: p=0.0012) (Figure 2, panel A and B). Birds that developed the most severe lesions were on average over 52 ± 12.758 g heavier just before *C. perfringens* challenge than birds that did not develop lesions. This effect of BW on lesion development was only observed for birds developing moderate and severe lesions, as no significant difference in BW before challenge was observed between birds without intestinal lesions as compared to the birds that developed mild NE lesions. Overall, a statistically significant, positive correlation was observed between the variables BW before challenge and lesion score (correlation coefficient 0.239 (p=<0.0001, n=283) at day 13 and 0.240 (p=<0.0001, n=285) at day 17).

The overall mean of the average daily BWG before *C. perfringens* challenge was 50.72 ± 0.75 g/day, 4.8% lower than the expected 53.25 g/day BWG for this period according to the Ross308 manual, confirming suboptimal conditions during the *in vivo* trial, which are used to predispose the birds to NE (Aviagen, 2014). The average daily BWG of the broilers during this 4-day period before *C. perfringens* challenge (day 13-17) was significantly higher for birds that developed severe NE lesions compared to birds that did not have any lesions (Figure 3). The latter showed on average 5.67 ± 1.92 g/day (12%) higher daily BWG than the birds without lesions (p=0.0030). Furthermore, birds that developed mild or moderate NE had respectively 2.76 ± 2.32 g/day (5.7%) or 2.67 ± 1.80 g/day (5.5%) higher average daily BWG before *C. perfringens* challenge than birds that did not develop NE lesions, which was not

statistically significant (no lesions vs. mild p=0.8702; no lesions vs. moderate p=0.1173). The correlation coefficient between the variables daily BWG before challenge and lesion score was 0.221 (p=0.002, n=281), indicating a statistically significant positive correlation.

Necrotic enteritis disease severity negatively affects daily body weight gain

In addition to the predisposing potential of high BW or higher daily BWG before *C. perfringens* challenge on the development of necrotic lesions, also the effect of NE disease severity on the evolution of BW and daily BWG during challenge was evaluated. After three days of *C. perfringens* challenge, no significant difference in BW could be observed between the birds that did not develop lesions as compared to birds that developed NE lesions of any severity (Figure 2, panel C).

According to the Ross308 performance manual, the average daily BWG of healthy birds between day 17 and 19 (corresponding to the *C. perfringens* challenge period in this study) increases by 20% as compared to its value between day 13 and 17 (corresponding to the period before challenge in this study) (Aviagen, 2014). However, when combining the data of all birds, the average daily BWG during *C. perfringens* challenge (day 17-19) was significantly lower than its value before challenge (day 13-17) (p<0.0001), indicating a reduction in growth rate due to *C. perfringens* challenge. This effect on average daily BWG was most pronounced in birds which developed severe necrotic lesions (Average daily BWG decreased from 54.28 ± 11.21 g/day before challenge to 26.88 ± 15.64 g/day during challenge, 51% reduction, p<0.0001). This effect lowered with decreasing disease severity (Average daily BWG for birds with moderate NE lesions decreased from 51.28 ± 11.45 g/day before challenge to 39.94 ± 13.54 g/day during challenge, 22% reduction, p=0.0073; Average daily BWG for birds with mild NE lesions decreased from 51.37 ± 17.01 g/day before challenge to 46.15 ± 12.84 g/day during challenge, 10% reduction, p=0.033). In birds that did not develop disease, no significant effect of *C. perfringens* challenge on average daily BWG was observed (Average daily BWG decreased from 48.61 ± 10.38 g/day before challenge to 46.05 ± 13.02 g/day during challenge, p=0.0657) (Figure 3).

In addition to the effect of *C. perfringens* challenge on the growth rate of the birds as compared to their growth rate before challenge, the average daily BWG during challenge was also compared between birds with different disease severity, irrespective of their growth rate before challenge. No difference in average daily BWG during the challenge period could be observed between birds that developed mild NE as compared to birds that did not develop lesions (Figure 3). However, the average daily BWG during *C. perfringens* challenge of both birds having moderate or severe lesions was significantly lower as compared to both birds without lesions or birds showing mild necrotic lesions. Birds suffering from moderate NE showed around 13% lower BWG than both the birds without lesions (Average daily BWG 46.05 ± 13.02 g/day for birds without lesions and 39.94 ± 13.51 g/day for birds with moderate lesions, $p=0.0361$) or birds showing mild lesions (Average daily BWG 46.15 ± 12.84 g/day for birds with mild lesions and 39.94 ± 13.51 g/day for birds with moderate lesions, $p=0.0451$). This difference in growth rate was even bigger for birds that developed severe lesions, with 42% less average daily BWG during challenge than birds that did not develop lesions (Average daily BWG 46.05 ± 13.02 g/day for birds without lesions and 26.88 ± 15.64 g/day for birds with severe lesions, $p<0.0001$) or developed only mild necrosis (Average daily BWG 46.15 ± 12.84 g/day for birds with mild lesions and 26.88 ± 15.64 g/day for birds with severe lesions, $p<0.0001$). The difference in average daily BWG for birds having moderate lesions as compared to birds having severe lesions was 33% (Average daily BWG 39.94 ± 13.51 g/day for birds with moderate lesions and 26.88 ± 15.64 g/day for birds with severe lesions 13.06 ± 2.891 g/day, $p=0.0021$). The correlation coefficient between the variables daily BWG during challenge and lesion score was -0.362 ($p<0.0001$, $n=281$), indicating a statistically significant negative correlation.

Discussion

Understanding the impact of predisposing factors to NE is crucial to come up with new strategies to tackle this economically important enteric disease. In this study, we clearly show that rapid growth increases the susceptibility of broilers to NE. Indeed, both BW and BWG before *C. perfringens* challenge were significantly greater for the birds that developed moderate to severe NE as compared to the birds that did not develop any lesions.

The observation that fast-growing animals tend to suffer more from *C. perfringens* related diseases is reported in various other production animal species (Lebrun et al., 2010) (Songer, 1996). However, the exact reason for this predisposition is still unclear. A first hypothesis is based on the immunological status of the host. Selection for high performing broilers has led to a decreased disease resistance, due to changes in both humoral and cellular immune responses (Cheema et al., 2003). Despite the significant effect between different breeds, the effect of rapid growth on the immune status of broilers within the same breed has not been assessed to date. Secondly, an effect of diet digestibility on predisposition to *C. perfringens* related diseases is commonly hypothesised. Diets rich in protein or non-starch polysaccharides are known to predispose to NE (Moore, 2016). Non-starch polysaccharides can also increase water intake, resulting in wet litter which is a known predisposing factor (McDevitt et al., 2006). In the current study all birds were supplied with the same high-energy diet, indicating that feed composition could not explain the observed effect. However, a rapid growth rate, demanding exceeding energy requirements has previously been associated with a higher feed intake (Howie et al., 2000). This might result in an increased amount of poorly digested proteins in more distal parts of the gut of faster growing birds, which can be used as a substrate for excessive *C. perfringens* proliferation (Williams et al., 2001) (Nakamura & Omaye, 2012). More broadly, this increased feed intake could result in microbial shifts, creating a favourable environment in which *C. perfringens* can proliferate. Indeed, there is a clear link of the gastrointestinal microbiota with both bird performance and the development of NE (Torok et al., 2011) (Johnson et al., 2018) (Stanley et al., 2012) (Stanley et al., 2016)

(Antonissen et al., 2016) (Wu et al., 2014). For example, overfeeding resulted in a reduced diversity and richness in the ileum in ducks, increasing the relative abundance of *Clostridiaceae* (Vasaï et al., 2014). Higher feed intake could also result in an increased coccidiosis incidence, indirectly affecting *C. perfringens* proliferation due to its predisposing nature to NE (Al-Sheikhly et al., 1979). Together, these hypotheses might explain the predisposing nature of a fast growth rate on NE disease severity observed in this study. Further studies are needed to confirm these hypotheses.

Skinner *et al.* (2010) observed that subclinical NE decreases BW at slaughter age by 12% compared to healthy broilers. Furthermore, a 25% BW reduction and 27% BWG reduction was described when comparing *C. perfringens* infected Cobb 500 broilers to non-infected birds (Latorre et al., 2018). Our results further support this data. Indeed, challenging broilers with *C. perfringens* during a three-day period (day 17-20) significantly reduced average daily BWG. This negative effect on growth rate was more pronounced with increased NE disease severity (reduction of 10% (mild), 22% (moderate) and 51% (severe) as compared to BWG before challenge). Although the broilers that developed moderate to severe NE were significantly heavier before challenge, their BW after *C. perfringens* challenge was not significantly different from that of broilers without lesions. These results confirm that NE is indeed of huge economic importance in which the advantage of a higher weight is lost after disease development.

The reduced growth rate observed in diseased birds might be explained by a reduced intestinal barrier function and inflammation in broilers which develop NE. Indeed, our research group has recently shown that broilers with severe necrotic enteritis suffer from intestinal barrier failure, whereas no intestinal leakage was measured in birds that did not develop disease or showed mild NE (Goossens et al., 2018). This is in accordance with the current study where, despite the large effect of *C. perfringens* challenge on both BW and BWG for birds that developed moderate to severe NE, no differences could be observed between birds without lesions and birds that developed only mild necrotic lesions. Together, these results confirm the intuitive notion that feed uptake by sick animals or nutrient uptake

by a moderate to severely damaged intestine is hampered, leading to reduced growth of the animals. The association between a higher growth rate and possible alterations in intestinal permeability have yet to be determined.

Based on the results described here, possible strategies to reduce NE susceptibility associated with a rapid growth rate can be proposed. Decreasing BWG by restricting feed consumption at a certain stage of the growth cycle could be used to contain NE occurrence. Tsiouris et al. (2014) described the positive effect of feed restriction on physico-chemical properties of the gut and its partial protection against subclinical NE in broilers. Feed restriction can lead to compensatory growth when feed consumption returns to its normal level, resulting in an equivalent slaughter weight but with a lower feed conversion ratio (Zubair & Leeson, 1996). Supplemental enzymes in the feed can also reduce the amount of undigested feed in the gut, altering the availability of nutrients to intestinal pathogens (Hajati et al., 2009) (McDevitt et al., 2006).

In conclusion, these results show an interplay between growth rate before *C. perfringens* challenge and NE disease severity, indicating that NE should be added to the list of production diseases (Julian, 2005). Controlling this disease will require an integrative approach. More research is needed to determine if limiting broiler performance can be part of a plan to prevent intestinal pathology, taking into account economic, ethical and practical consequences during implementation.

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350

Figure legends

Figure 1: Timeline of the necrotic enteritis *in vivo* experiment.

Predisposing factors include oral administration of Nobilis Gumboro D78 vaccine at days 4 and 9, oral administration of a tenfold dose of Hipracox at day 14 and a tenfold dose of Paracox-5 at day 16. Fishmeal was added to the feed from day 17 onwards. All broilers were challenged with *C. perfringens* (Black bar), resulting in the induction of subclinical NE. Here for, at days 17, 18 and 19, one millilitre overnight culture of the pathogenic *C. perfringens* strain CP56 was orally administered. At day 20, birds were euthanized. Birds were weighed at days 13, 17 and 20.

Figure 2: Body weight of broilers at different time points during a necrotic enteritis *in vivo* trial.

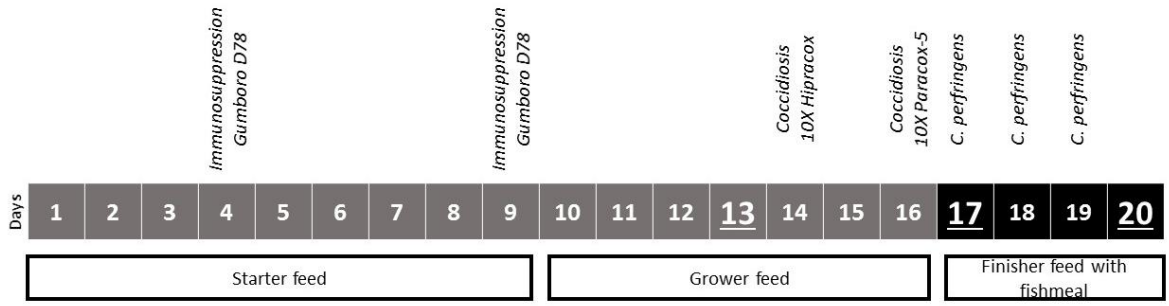
The birds were challenged with *C. perfringens* on days 17, 18 and 19 after which the severity of necrotic lesions was determined. The graph represents the average body weight of the broilers (+- SEM) grouped by final disease severity at: (A) 4 days before challenge (day 13), (B) one hour before challenge (day 17) and (C) after challenge (day 20); no lesions (score 0), mild (score 2), moderate (scores 3-4) and severe (score 5-6). Groups with different letters are significantly different at a significance level of 5%.

Figure 3: Average daily body weight gain of broilers before and during challenge during a necrotic enteritis *in vivo* trial.

The birds were challenged with *C. perfringens* on days 17, 18 and 19 after which the severity of necrotic lesions was determined. The graph represents the average daily body weight gain of the broilers (+- SEM) grouped by final disease severity before *C. perfringens* challenge (day 13-17; black bars) and

374 during *C. perfringens* challenge (day 17-20; white bars); no lesions (score 0), mild (score 2), moderate
375 (scores 3-4) and severe (score 5-6). The average daily body weight gain is calculated as the (weight at
376 day 17 – weight at day 13)/4 days and (weight at day 20 – weight at day 17)/3 days. Statistically
377 significant differences (at a significance level of 5%) between groups based on BWG before challenge,
378 during challenge or comparing BWG before versus after challenge are depicted using different letters,
379 letters with apostrophe and asterisk symbols, respectively (*= $p<0.05$, **= $p<0.01$ ***= $p<0.001$).

Figures



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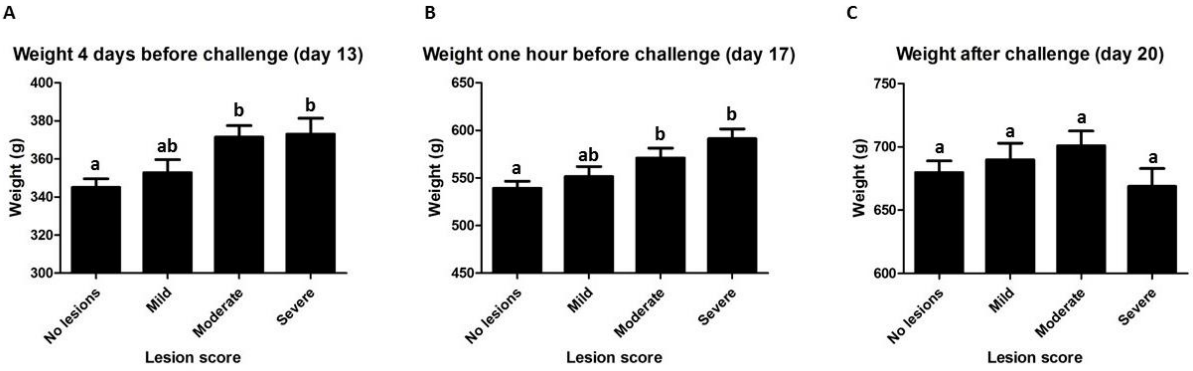

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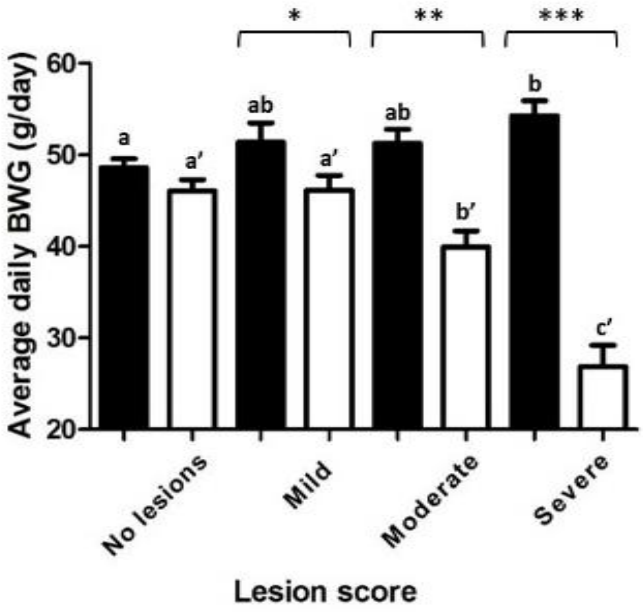

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397 *birds were challenged with C. perfringens on days 17, 18 and 19 after which the severity of necrotic lesions was determined.*
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399 *perfringens challenge (day 13-17; black bars) and during C. perfringens challenge (day 17-20; white bars); no lesions (score*
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401 *at day 17 – weight at day 13)/4 days and (weight at day 20 – weight at day 17)/3 days. Statistically significant differences (at*
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