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1 Rapid growth predisposes broilers to necrotic enteritis

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1 **Abstract**

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

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

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17 **Research highlights**

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

## 1 Introduction

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

12

13 Despite its clear economic advantages, rapidly increasing BWG has a high burden on animal health,  
14 leading to an increase in metabolic, musculoskeletal and cardiovascular disorders in broilers with the  
15 highest productivity (Angel, 2007) (Lilburn, 1994) (Kestin et al., 1992) (Rath et al., 2018) (Williams et  
16 al., 2004) (Kumari et al., 2016) (Scheele, 1997) (Peacock et al., 1990) (Olkowski, 2006) (Mazzoni et al.,  
17 2015) (Kuttappan et al., 2016). In contrast to the effect of increased BWG on susceptibility to metabolic  
18 diseases, the impact on infectious diseases is less well described but plausible. Cheema et al. (2003)  
19 describes the alteration of the immune response to pathogens in fast-growing broilers, resulting in a  
20 decline in disease resistance.

21

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

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

42

#### 43 **Keywords**

44 Necrotic enteritis; Broiler; Body weight gain; Predisposing factor

45 **Material and Methods**

46 ***Necrotic enteritis in vivo trial***

47 Three hundred broilers (mixed sex) were housed in the same stable. A well-established NE challenge  
48 model was used with minor modifications (Gholamiandehkordi et al., 2007). Figure 1 represents a  
49 timeline of the NE *in vivo* protocol. In short, water and feed were supplied ad libitum. The feed was a  
50 wheat/rye-based (43%/7.5%) diet containing soybean meal as a protein source. At 17 days of age, the  
51 soybean meal was replaced by fishmeal (30%), increasing the amount of animal protein which is a  
52 predisposing factor for induction of NE. A mild form of immunosuppression was induced by oral  
53 administration of an infectious bursal disease vaccine (Nobilis Gumboro D78 vaccine, Schering-Plough  
54 Animal Health, Brussels, Belgium) at days 4 and 9. Mild intestinal damage was induced through oral  
55 administration of a tenfold dose of a live coccidiosis vaccine at day 14 and day 16 (day 14: Hipracox,  
56 Hipra, Gerona, Spain; day 16: Paracox-5, MSD Schering-Plough Animal Health, Brussels, Belgium). All  
57 broilers were challenged with a well-characterized NetB+ *C. perfringens* (CP56, Gholamiandehkordi et  
58 al., 2007), resulting in the induction of subclinical NE. Here for, at days 17, 18 and 19, one millilitre  
59 overnight culture (in Brain heart infusion broth (Bio-Rad, Temse, Belgium, product number 64014)) of  
60 the pathogenic *C. perfringens* strain CP56 was orally administered to each bird. At day 20, birds were  
61 euthanized. The experiments were carried out according to the recommendations and following  
62 approval from the Ethical Committee of the faculty of Veterinary Medicine at Ghent University  
63 (EC2016\_127).

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

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

## 72 ***Statistical analysis***

73 In order to gain more insight into the link between BW or average daily BWG and NE disease severity,  
74 the data of the NE trial were grouped according to disease severity. The data were divided into 4  
75 groups: No lesions (score 0; n=117), Mild (score 2; n=67), Moderate (score 3 and 4; n=66) and Severe  
76 (score 5 and 6; n=50). The results were analysed using Graphpad Prism 8. Normality was checked using  
77 the D'Agostino and Pearson omnibus normality test. The non-parametric Kruskal-Wallis test was used  
78 to compare the means of BW or average daily BWG between groups (No lesions, Mild, Moderate and  
79 Severe). A pairwise comparison was made using a Dunn correction at an overall significance level of  
80 5%. The given p-values were adjusted to the multiple pairwise comparison. Quantitative differences  
81 between means of groups are given with their corresponding SEM value, calculated using the baseline  
82 correction function of GraphPad Prism. A two-tailed paired t-test was used to compare BWG before  
83 and after *C. perfringens* challenge. The correlation coefficient was obtained using the Spearman  
84 correction method.

85

86 **Results**

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

89 The effect of the initial BW of the birds before *C. perfringens* challenge on the outcome of necrotic  
90 enteritis lesion development was evaluated both at 4 days before (day 13) and one hour before (day  
91 17) *C. perfringens* challenge. When comparing the BW of birds that did not develop NE to birds that  
92 developed moderate to severe NE, a significant difference could be observed both at four days before  
93 (no lesions vs. moderate NE:  $p=0.0044$ ; no lesions vs. severe NE:  $p=0.0072$ ) and at one hour before *C.*  
94 *perfringens* challenge (no lesions vs. moderate NE:  $p=0.0362$ ; no lesions vs. severe NE:  $p=0.0012$ )  
95 (Figure 2, panel A and B). Birds that developed the most severe lesions were on average over  $52 \pm$   
96  $12.758$  g heavier just before *C. perfringens* challenge than birds that did not develop lesions. This effect  
97 of BW on lesion development was only observed for birds developing moderate and severe lesions, as  
98 no significant difference in BW before challenge was observed between birds without intestinal lesions  
99 as compared to the birds that developed mild NE lesions. Overall, a statistically significant, positive  
100 correlation was observed between the variables BW before challenge and lesion score (correlation  
101 coefficient  $0.239$  ( $p < 0.0001$ ,  $n=283$ ) at day 13 and  $0.240$  ( $p < 0.0001$ ,  $n=285$ ) at day 17).

102 The overall mean of the average daily BWG before *C. perfringens* challenge was  $50.72 \pm 0.75$  g/day,  
103 4.8% lower than the expected  $53.25$  g/day BWG for this period according to the Ross308 manual,  
104 confirming suboptimal conditions during the *in vivo* trial, which are used to predispose the birds to NE  
105 (Aviagen, 2014). The average daily BWG of the broilers during this 4-day period before *C. perfringens*  
106 challenge (day 13-17) was significantly higher for birds that developed severe NE lesions compared to  
107 birds that did not have any lesions (Figure 3). The latter showed on average  $5.67 \pm 1.92$  g/day (12%)  
108 higher daily BWG than the birds without lesions ( $p=0.0030$ ). Furthermore, birds that developed mild  
109 or moderate NE had respectively  $2.76 \pm 2.32$  g/day (5.7%) or  $2.67 \pm 1.80$  g/day (5.5%) higher average  
110 daily BWG before *C. perfringens* challenge than birds that did not develop NE lesions, which was not

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

114

#### 115 ***Necrotic enteritis disease severity negatively affects daily body weight gain***

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

121 According to the Ross308 performance manual, the average daily BWG of healthy birds between day  
122 17 and 19 (corresponding to the *C. perfringens* challenge period in this study) increases by 20% as  
123 compared to its value between day 13 and 17 (corresponding to the period before challenge in this  
124 study) (Aviagen, 2014). However, when combining the data of all birds, the average daily BWG during  
125 *C. perfringens* challenge (day 17-19) was significantly lower than its value before challenge (day 13-17)  
126 ( $p<0.0001$ ), indicating a reduction in growth rate due to *C. perfringens* challenge. This effect on average  
127 daily BWG was most pronounced in birds which developed severe necrotic lesions (Average daily BWG  
128 decreased from  $54.28 \pm 11.21$  g/day before challenge to  $26.88 \pm 15.64$  g/day during challenge, 51%  
129 reduction,  $p<0.0001$ ). This effect lowered with decreasing disease severity (Average daily BWG for  
130 birds with moderate NE lesions decreased from  $51.28 \pm 11.45$  g/day before challenge to  $39.94 \pm 13.54$   
131 g/day during challenge, 22% reduction,  $p=0.0073$ ; Average daily BWG for birds with mild NE lesions  
132 decreased from  $51.37 \pm 17.01$  g/day before challenge to  $46.15 \pm 12.84$  g/day during challenge, 10%  
133 reduction,  $p=0.033$ ). In birds that did not develop disease, no significant effect of *C. perfringens*  
134 challenge on average daily BWG was observed (Average daily BWG decreased from  $48.61 \pm 10.38$  g/day  
135 before challenge to  $46.05 \pm 13.02$  g/day during challenge,  $p=0.0657$ ) (Figure 3).

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

157 **Discussion**

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

163 The observation that fast-growing animals tend to suffer more from *C. perfringens* related diseases is  
164 reported in various other production animal species (Lebrun et al., 2010) (Songer, 1996). However, the  
165 exact reason for this predisposition is still unclear. A first hypothesis is based on the immunological  
166 status of the host. Selection for high performing broilers has led to a decreased disease resistance, due  
167 to changes in both humoral and cellular immune responses (Cheema et al., 2003). Despite the  
168 significant effect between different breeds, the effect of rapid growth on the immune status of broilers  
169 within the same breed has not been assessed to date. Secondly, an effect of diet digestibility on  
170 predisposition to *C. perfringens* related diseases is commonly hypothesised. Diets rich in protein or  
171 non-starch polysaccharides are known to predispose to NE (Moore, 2016). Non-starch polysaccharides  
172 can also increase water intake, resulting in wet litter which is a known predisposing factor (McDevitt  
173 et al., 2006). In the current study all birds were supplied with the same high-energy diet, indicating  
174 that feed composition could not explain the observed effect. However, a rapid growth rate, demanding  
175 exceeding energy requirements has previously been associated with a higher feed intake (Howie et al.,  
176 2000). This might result in an increased amount of poorly digested proteins in more distal parts of the  
177 gut of faster growing birds, which can be used as a substrate for excessive *C. perfringens* proliferation  
178 (Williams et al., 2001) (Nakamura & Omaye, 2012). More broadly, this increased feed intake could  
179 result in microbial shifts, creating a favourable environment in which *C. perfringens* can proliferate.  
180 Indeed, there is a clear link of the gastrointestinal microbiota with both bird performance and the  
181 development of NE (Torok et al., 2011) (Johnson et al., 2018) (Stanley et al., 2012) (Stanley et al., 2016)

182 (Antonissen et al., 2016) (Wu et al., 2014). For example, overfeeding resulted in a reduced diversity  
183 and richness in the ileum in ducks, increasing the relative abundance of *Clostridiaceae* (Vasai et al.,  
184 2014). Higher feed intake could also result in an increased coccidiosis incidence, indirectly affecting *C.*  
185 *perfringens* proliferation due to its predisposing nature to NE (Al-Sheikhly et al., 1979). Together, these  
186 hypotheses might explain the predisposing nature of a fast growth rate on NE disease severity  
187 observed in this study. Further studies are needed to confirm these hypotheses.

188 Skinner *et al.* (2010) observed that subclinical NE decreases BW at slaughter age by 12% compared to  
189 healthy broilers. Furthermore, a 25% BW reduction and 27% BWG reduction was described when  
190 comparing *C. perfringens* infected Cobb 500 broilers to non-infected birds (Latorre et al., 2018). Our  
191 results further support this data. Indeed, challenging broilers with *C. perfringens* during a three-day  
192 period (day 17-20) significantly reduced average daily BWG. This negative effect on growth rate was  
193 more pronounced with increased NE disease severity (reduction of 10% (mild), 22% (moderate) and  
194 51% (severe) as compared to BWG before challenge). Although the broilers that developed moderate  
195 to severe NE were significantly heavier before challenge, their BW after *C. perfringens* challenge was  
196 not significantly different from that of broilers without lesions. These results confirm that NE is indeed  
197 of huge economic importance in which the advantage of a higher weight is lost after disease  
198 development.

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

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

210

211 Based on the results described here, possible strategies to reduce NE susceptibility associated with a  
212 rapid growth rate can be proposed. Decreasing BWG by restricting feed consumption at a certain stage  
213 of the growth cycle could be used to contain NE occurrence. Tsiouris et al. (2014) described the positive  
214 effect of feed restriction on physico-chemical properties of the gut and its partial protection against  
215 subclinical NE in broilers. Feed restriction can lead to compensatory growth when feed consumption  
216 returns to its normal level, resulting in an equivalent slaughter weight but with a lower feed conversion  
217 ratio (Zubair & Leeson, 1996). Supplemental enzymes in the feed can also reduce the amount of  
218 undigested feed in the gut, altering the availability of nutrients to intestinal pathogens (Hajati et al.,  
219 2009) (McDevitt et al., 2006).

220 In conclusion, these results show an interplay between growth rate before *C. perfringens* challenge  
221 and NE disease severity, indicating that NE should be added to the list of production diseases (Julian,  
222 2005). Controlling this disease will require an integrative approach. More research is needed to  
223 determine if limiting broiler performance can be part of a plan to prevent intestinal pathology, taking  
224 into account economic, ethical and practical consequences during implementation.

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350

351 **Figure legends**

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

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

359

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

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

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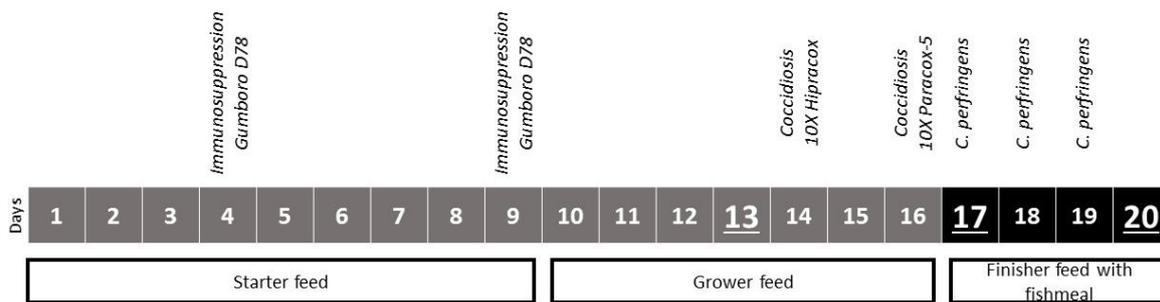
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369 **Figure 3: Average daily body weight gain of broilers before and during challenge during a necrotic  
370 enteritis *in vivo* trial.**

371 The birds were challenged with *C. perfringens* on days 17, 18 and 19 after which the severity of necrotic  
372 lesions was determined. The graph represents the average daily body weight gain of the broilers (+-  
373 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).

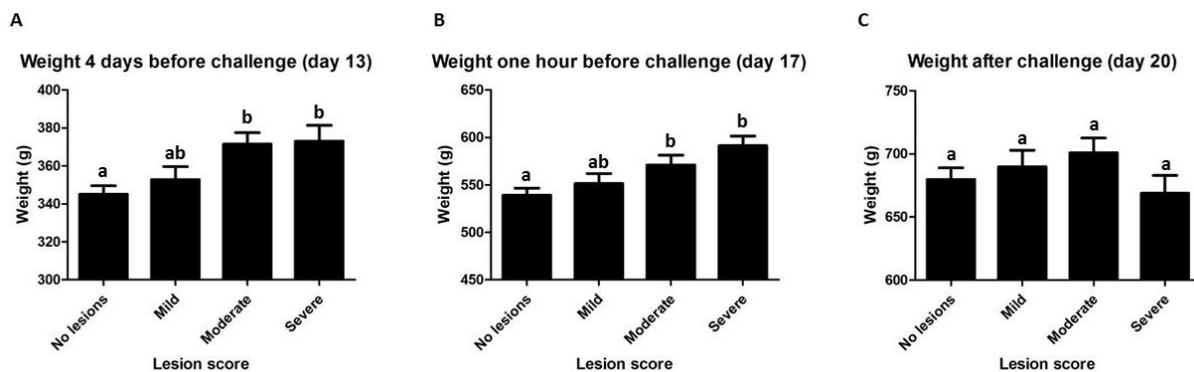
380 **Figures**



381

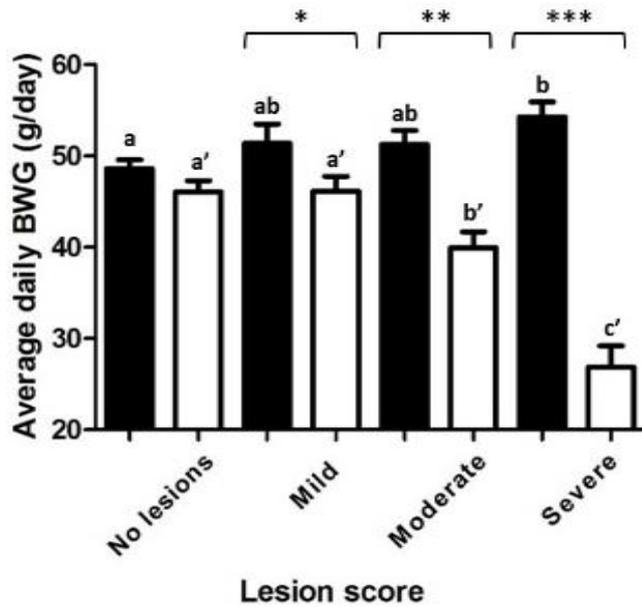
382 *Figure 1: Timeline of the necrotic enteritis in vivo experiment. Predisposing factors include oral administration of Nobilis*  
 383 *Gumboro D78 vaccine at days 4 and 9, oral administration of a tenfold dose of Hipracox at day 14 and a tenfold dose of*  
 384 *Paracox-5 at day 16. Fishmeal was added to the feed from day 17 onwards. All broilers were challenged with C. perfringens*  
 385 *(Black bar), resulting in the induction of subclinical NE. Here for, at days 17, 18 and 19, one millilitre overnight culture of the*  
 386 *pathogenic C. perfringens strain CP56 was orally administered. At day 20, birds were euthanized. Birds were weighed at days*  
 387 *13, 17 and 20.*

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389

390 *Figure 2: Body weight of broilers at different time points during a necrotic enteritis in vivo trial. The birds were challenged with*  
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 393 *hour before challenge (day 17) and (C) after challenge (day 20); no lesions (score 0), mild (score 2), moderate (scores 3-4) and*  
 394 *severe (score 5-6). Groups with different letters are significantly different at a significance level of 5%.*



395

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 398 *The graph represents the average daily body weight gain of the broilers (+ SEM) grouped by final disease severity before C.*  
 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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 404 *p<0.01 \*\*\*= p<0.001).*