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# Effects of dietary inclusion of chromium propionate on growth performance, intestinal health, immune response and nutrient transporter gene expression in broilers

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1 **Effects of dietary inclusion of chromium propionate on growth performance, intestinal**  
2 **health, immune response and nutrient transporter gene expression in broilers**

3

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21 **Abstract**

22 The intent of the present study was to evaluate the effect of chromium ( $\text{Cr}^{3+}$ ) as chromium  
23 propionate on growth performance, organ index, immune response, intestinal morphology and  
24 nutrient transporter gene expression in broilers. One-day-old broiler chicks ( $n=756$ ) were divided  
25 into six experimental groups of 126 chicks; each group was further divided into 7 replicates (18  
26 chicks/replicate). All birds were offered corn-soybean diets supplemented with  $\text{Cr}^{3+}$  at 0, 0.10,  
27 0.15, 0.20, 0.25 or 0.30 mg/kg. Dietary inclusion of  $\text{Cr}^{3+}$  at various levels yielded significantly  
28 better growth performance and organ index in birds. Similarly, antibody titre against Newcastle  
29 disease and avian influenza H5 at various ages was found to be significantly higher in birds that  
30 received 0.15 mg/kg  $\text{Cr}^{3+}$  in the diet. Significant results with respect to villus height (VH), crypt  
31 depth (CD) and VH:CD were observed in all groups that received  $\text{Cr}^{3+}$  in the diet compared to  
32 control. Moreover, it was observed that different levels of  $\text{Cr}^{3+}$  supplementation of the diet also  
33 increased the expression of the nutrient transporter genes SGLT1, GLUT2, rBAT and CAT1 in  
34 broilers. The findings of the present study suggest that dietary inclusion of  $\text{Cr}^{3+}$  at various levels  
35 may have beneficial effects on growth performance, immunity, intestinal morphology and  
36 nutrient transporter gene expression in broilers. Supplementation of the diet with  $\text{Cr}^{3+}$  at a level  
37 of 0.15 mg/kg could yield better performance in broiler production.

38

39 **Key words:** broilers; chromium; growth performance; organ index; antibody titre; gene  
40 expression

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## 42        **1. Introduction**

43        With the rapid development of the poultry industry, scientists around the globe are now focusing  
44        on the role of micronutrients in poultry diets to ensure better production. Although  
45        micronutrients are required in minute quantities in the poultry diet, they play an important role in  
46        improving birds' health and vigour. In recent decades, the use of Cr in the nutrition of domestic  
47        animals has attracted the attention of researchers. Chromium can be present in the environment  
48        at numerous oxidation states, with  $\text{Cr}^{3+}$  being the most stable form (NRC, USA, 2005).  
49        Chromium is a micronutrient that is involved in the metabolism of carbohydrates, proteins, lipids  
50        and nucleic acids (Khan et al., 1994; Sands and Smith, 1999). Chromium activates many of the  
51        enzymes responsible for protein and nucleic acid synthesis (Anderson, 1994). Dietary  
52        supplementation with  $\text{Cr}^{3+}$  is recommended by the National Research Council (NRC)  
53        Washington, USA for animals suffering from environmental stress (NRC, 1997). However,  $\text{Cr}^{3+}$   
54        supplementation poses a challenge because the dose must be adjusted to provide the actual  
55        needed concentration to avoid hepatotoxic, nephrotoxic, oxidative and DNA-damaging effects  
56        (Deng et al., 2015; Jin et al., 2015). Studies provide indications for the nutritional and  
57        physiological role of  $\text{Cr}^{3+}$  as a micronutrient (Anderson, 1994). The beneficial effects of  $\text{Cr}^{3+}$  are  
58        most obvious in animals that are under environmental, dietary and hormonal stress because  $\text{Cr}^{3+}$   
59        can reduce the harmful effects of stress caused by various environmental factors (Lien et al.,  
60        1999; Sahin et al., 1992). Some studies have shown that chromium supplementation of broiler  
61        feed positively affects the growth performance and carcass characteristics of birds (Debski et al.,  
62        2004; Toghyani et al., 2006; Sahin et al., 2010; Huang et al., 2016). However, other studies  
63        reported opposite results (Moeini et al., 2011; Ghazi et al., 2012; Kim et al., 1996).

64 At present, poultry diseases represent the major threat to the poultry industry. Newcastle disease  
65 virus (NDV), a **virus that causes a deadly infection**, infects many bird species and is highly  
66 contagious and fatal to susceptible birds (Alexander et al., 1998). NDV, which belongs to the  
67 genus *Avulavirus* and the family Paramyxoviridae, is the causative agent of a respiratory and  
68 neurological disease (Newcastle disease) that can cause up to 100% morbidity and mortality in  
69 infected birds (Alexander, 2003). Newcastle disease is primarily controlled by maintaining a  
70 hygienic environment and a good vaccination program. Currently, vaccination against many  
71 diseases is helpful in controlling severe sickness and mortality in infected birds (Van Boven et  
72 al., 2008). Recently, lentogenic strains of NDV have been used to induce a protective immune  
73 response and studies suggest that different vaccine strategies have specific advantages and  
74 disadvantages (Bermudez and Stewart, 2003). Chromium can also increase the immune status of  
75 broilers by overwhelming the production of corticosterone, a potent immunosuppressant that  
76 interferes with antibody production by decreasing leukocyte populations and interfering with  
77 lymphocyte function (Sahin et al., 2005; Lee et al., 2003). To determine the usefulness of  
78 chromium addition in producing a good-quality poultry diet that improves birds' performance  
79 and resistance to diseases, the present study assessed the effect of dietary Cr<sup>3+</sup> on growth  
80 performance, organ index, immune response, histomorphology and nutrient transporter gene  
81 expression in broilers.

82

## 83 **2. Materials and methods**

### 84 **2.1. Experimental birds and diet**

85 The present study was conducted at the poultry farm of North West Agriculture and Forestry  
86 University, Yangling, China. Throughout the experiment, the birds were maintained under

87 constant conditions of temperature, relative humidity, ventilation, light cycle and floor space.  
88 The 756 one-day-old broiler chicks used in this experiment were randomly divided into six  
89 experimental groups (A, B, C, D, E and F). Chromium (dry, 0.4%) from chromium propionate is  
90 designed to be premixed prior to its addition to complete broiler feed. Premix contain 10%  
91 chromium (from 0.4% chromium dry) and 90% calcium carbonate (having 35% calcium)  
92 delivered Cr @ 0, 0.10, 0.15, 0.20, 0.25 or 0.30 mg/kg in the feed. Each group of 126 chicks was  
93 further divided into 7 replicates (18 chicks/replicate). The birds were fed a corn-soybean diet that  
94 was formulated to meet all nutritional requirements according to the feeding standard in China  
95 (NY/T33-2004) (Table 6).

## 96 **2.2. Experimental records and management**

97 One-day-old birds were weighed, and the average initial body weight of the birds in each group  
98 was calculated. The birds were provided with feed and water *ad libitum* throughout the  
99 experiment. The number of dead birds and the temperature and humidity in the birdhouse were  
100 recorded on a daily basis. Management requirements and data were recorded regularly. The birds  
101 were vaccinated according to routine immunization practice in the region. The broiler house was  
102 kept clean and was disinfected on a regular basis.

## 103 **2.3. Clinical observation**

104 During the trial period, the clinical features of the broilers, including their consumption of food  
105 and water, were observed daily and bad symptoms (abnormal health conditions or mortality), if  
106 any, were noted. All dead birds were dissected, and a detailed post-mortem was conducted.

## 107 **2.4. Growth performance**

108 At 0, 21 and 42 days, the birds were weighed, and the feed intake of each replicate was recorded.  
109 Average daily feed intake (ADFI), average daily weight gain (ADWG), feed conversion ratio  
110 (FCR) and mortality were calculated for the periods 0-21 and 0-42 days.

111

## 112 **2.5. Organ index**

113 Seven birds from each treatment group whose weights were close to the average body weights  
114 (birds having minimum difference in body weight to average weight of their respective  
115 treatment) of the birds in their respective groups were selected at the age of 21 and 42 days and  
116 deprived of feed for 12 h to determine weight loss during the pre-slaughter holding period. The  
117 pre-slaughter body weight loss was expressed as g/kg initial body weight of each individual bird.  
118 Later in the study, these birds were slaughtered, and the organ index (relative organ weight of the  
119 liver, heart, spleen and thymus) was recorded and expressed as g/kg pre-slaughter live body  
120 weight of the individual birds.

## 121 **2.6. Immune response**

122 The birds were tested for serum antibody titres on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days after  
123 vaccination against Newcastle disease and on the 28<sup>th</sup> day after vaccination against avian  
124 influenza H5 by HI test according to a standard protocol (OIE, 2012).

## 125 **2.7. Intestinal morphology**

126 At 21 and 42 days of age, seven birds from each treatment group (1 bird/replicate) whose body  
127 weights were close to the average body weights of their respective groups were selected. The  
128 birds were slaughtered, and the duodenum, jejunum and ileum were removed and cleaned by  
129 flushing with pre-cooled saline. Sections (approximately 1 cm in length) were cut from the

130 middle of the duodenum, jejunum and ileum and fixed in 4% paraformaldehyde. The villus  
131 height (VH), the crypt depth (CD) and the thickness of the intestinal wall (WT) were measured.

## 132 **2.8. RNA isolation and quantitative real - time PCR**

133 The mRNA expression of SGLT1, GLUT2, rBAT, and CAT1 in intestinal mucosal samples from  
134 the duodenum, jejunum and ileum was determined by qRT - PCR. The primers used in these  
135 experiments (Table 7) were designed using Premier 5.0 according to sequences obtained from  
136 NCBI. Total RNA was isolated using trizol reagent according to the manufacturer's instructions  
137 (Invitrogen, Carlsbad, CA, USA). The quantity and quality of the extracted RNA in each sample  
138 were determined using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Inc.,  
139 Wilmington, DE, USA). The RNA was stored at  $-20^{\circ}\text{C}$  until further use. The RNA was  
140 reverse - transcribed using a Takara cDNA transfer kit (Bio - Rad, Hercules, CA, USA). The  
141 reaction was conducted in a total volume of 20  $\mu\text{L}$  consisting of 1  $\mu\text{L}$  cDNA, 1  $\mu\text{L}$  forward  
142 primer, 1  $\mu\text{L}$  reverse primer, 10  $\mu\text{L}$  SYBR Premix ExTaq and 7  $\mu\text{L}$  nuclease - free water. The  
143 qPCR was conducted in triplicate using 96 - well microwell plates in a Bio - Rad iQ5 Real -  
144 Time PCR Detection System (Bio - Rad, Hercules, CA, USA). The PCR conditions were as  
145 follows:  $95^{\circ}\text{C}$  for 5 min followed by 40 cycles of  $95^{\circ}\text{C}$  for 10 s,  $60^{\circ}\text{C}$  for 30 s and  $72^{\circ}\text{C}$  for 30 s  
146 and a final extension at  $72^{\circ}\text{C}$  for 5 min. The mRNA expression of each gene was calculated  
147 using the  $2^{-\Delta\Delta\text{Ct}}$  method described by Livak and Schmittgen (2001) after normalizing to  $\beta$  -  
148 actin.

## 149 **2.9. Statistical analysis**



150 The experimental data were analysed by one-way ANOVA using SPSS 20.0 (SPSS Inc.,  
151 Chicago, IL, USA). A probability value of  $P < 0.05$  was considered to indicate statistical  
152 significance. The notable differences between groups were identified by Duncan's multiple  
153 comparisons test.

### 154 **3. Results**

#### 155 **3.1. Growth Performance**

156  $\text{Cr}^{3+}$  supplementation of the birds' feed resulted in significantly higher ADFI in group B (dietary  
157 supplementation with  $\text{Cr}^{3+}$  at 0.10 mg/kg), followed by groups C, A, F, D and E, in that order, at  
158 1-21 days of age. ADWG was significantly higher in the birds in groups B and C (dietary  
159 supplementation with  $\text{Cr}^{3+}$  at 0.10 and 0.15 mg/kg, respectively) compared to the other groups at  
160 1-21 and 1-42 days of age, respectively. Moreover, the feed conversion ratio was found to be  
161 significantly better in group C (dietary supplementation with  $\text{Cr}^{3+}$  at 0.15 mg/kg) than in any of  
162 the other groups, followed by groups D, E, F, B and A, in that order, at 1-21 days of age. At 1-42  
163 days of age, the feed conversion ratio was found to be significantly ( $P < 0.05$ ) better in all  
164 chromium-supplemented groups compared to the control. The best FCR was observed in group  
165 C, followed by groups B, D, E, F and A (figure 1).

#### 166 **3.2. Organ Index**

167 The birds in group B, which were offered a diet supplemented with  $\text{Cr}^{3+}$  at 0.10 mg/kg, showed a  
168 significantly increased spleen index at 21 days of age compared with the birds in the control and  
169 D, E and F groups ( $P < 0.05$ ). The birds in group F, which received a diet supplemented with  $\text{Cr}^{3+}$   
170 at 0.30 mg/kg, showed a significantly increased thymus index at 21 days of age. At 42 days of  
171 age, it was found that the birds in group D, which received a diet supplemented with  $\text{Cr}^{3+}$  at 0.15  
172 mg/kg, had significantly higher heart index compared to the control and to all other treatment

173 groups. Groups C and F, whose feed was supplemented with Cr<sup>3+</sup> at 0.15 mg/kg and 0.30 mg/kg,  
174 respectively, showed significantly better thymus index results compared with the control and  
175 with groups B and E (Table 1).

#### 176 **a. Immune response**

177 The birds in treatment group C, which were offered a diet supplemented with 0.15 mg/kg Cr<sup>3+</sup>,  
178 showed significantly higher antibody titres than the control group and group E on day 7 after  
179 immunization against Newcastle disease. At 21 days after immunization, the birds in groups C  
180 and D showed significantly higher antibody titres against Newcastle disease than the control  
181 group and group E; at 28 days after immunization, they showed significantly higher antibody  
182 titres than the control group and group B. The antibody titre against H5 at 21 days after  
183 immunization was found to be significantly higher in the birds in group D than in the other  
184 treatment groups, although it was not significantly different from the control (Table 2).

#### 185 **b. Intestinal morphology**

186 Figures 2-4 show the intestinal morphology of the animals. The results presented in Tables 3-5  
187 show that the birds in group C, which received a diet supplemented with Cr<sup>3+</sup> at 0.15 mg/kg, had  
188 significantly increased crypt depth compared to the control group. Groups C and F showed  
189 significantly increased WT in the jejunum compared to all of the other groups at 21 days of age .  
190 In addition, the birds in groups B, C, D and E, which received diets supplemented with Cr<sup>3+</sup> at  
191 0.10, 0.15, 0.20 and 0.25 mg/kg, respectively, showed a significantly reduced VH:CD ratio in the  
192 jejunum at 21 days of age (P <0.05).

#### 193 **c. Glucose and amino acid transporter gene expression**

194 Dietary supplementation with Cr<sup>3+</sup> significantly increased the relative expression of the SGLT1  
195 gene in the duodenum of broilers at 21 days of age. The highest expression of the SGLT1 gene

196 was observed in group F (dietary supplementation with 0.30 mg/kg Cr<sup>3+</sup>), followed by groups D,  
197 C, E, B and A. At 42 days of age, CAT1 mRNA gene expression was highest in group D  
198 (dietary supplementation with 0.20 mg/kg Cr<sup>3+</sup>), followed by groups F, A, B, E and A. In the  
199 jejunum, CAT1 mRNA expression was significantly higher in group C (dietary supplementation  
200 with 0.15 mg/kg Cr<sup>3+</sup>) than in any of the other groups, while at 42 days of age GLUT2 mRNA  
201 expression was significantly higher in all of the chromium-supplemented groups than in the  
202 control. However, at 42 days of age, CAT1 mRNA showed significantly higher expression in  
203 the jejunum in groups C and F compared to all other groups. In the ileum at 21 days of age,  
204 GLUT2 mRNA expression was significantly higher in group C (dietary supplementation with  
205 0.15 mg/kg Cr<sup>3+</sup>) than in any of the other groups, whereas CAT1 expression was significantly  
206 higher in groups B and C compared to all other groups. At 42 days of age, GLUT2 expression in  
207 the ileum was highest in group C, followed by groups D, B, F, E and A, and CAT1 expression  
208 was significantly higher ( $P < 0.05$ ) in group C compared to all the other groups (figure 5).

#### 209 **4. Discussion**

210 In the present study, birds were reared under normal conditions without exposure to obvious  
211 stress factors. The results indicate that supplementation of the diet with Cr<sup>3+</sup> at various  
212 concentrations had significant effects on the growth performance (ADFI, ADWG and FCR) of  
213 broilers at different ages. Dietary supplementation with Cr<sup>3+</sup> significantly increased ADFI in the  
214 birds in group B at 1-21 days of age. ADWG was significantly higher in the birds in groups B  
215 and C than in those in the other groups at 1-21 and 1-42 days of age. Moreover, the feed  
216 conversion ratio was found to be significantly better in group C than in any of the other groups at  
217 1-21 days of age. At 1-42 days of age, the feed conversion ratio was significantly better in all  
218 chromium-supplemented groups than in the control. Best FCR was obtained in group C. These

219 results are consistent with the results of some previous studies. Toghiani et al (2006) reported  
220 that dietary supplementation with CrPic at increased levels (up to 1,500  $\mu\text{g}/\text{kg}$ ) enhanced body  
221 weight and feed intake without any adverse effect on FCR in broilers reared under heat stress.  
222 Sahin et al (2010) found a beneficial effect of CrPic on body weight gain in quail. Huang et al  
223 (2016) found that supplementation of the diet with chromium improved the dressing  
224 percentage of broilers reared under heat stress. Afrin et al (2016) found that dietary  
225 chromium supplementation significantly increased body weight and feed intake in broilers.  
226 Kheiri and Toghiani (2009) reported that chromium supplementation of the diet significantly  
227 increased body weight in broilers without altering feed intake or FCR. Naghieh et al. (2010)  
228 found that the feed intake and body weight of broilers offered a diet supplemented with  $\text{Cr}^{3+}$   
229 were significantly increased. El-Kholy et al. (2017) reported that inclusion of chromium in the  
230 diet increased the daily body weight gain of broilers reared under heat stress conditions without  
231 affecting overall growth performance. However, the results of some other studies are contrary to  
232 those of the present study. It was reported that dietary addition of organic and inorganic  $\text{Cr}^{3+}$  at  
233 different levels resulted in no difference in weight gain or feed intake in heat- stressed broilers  
234 (Moeini et al., 2011). In another study, it was found that supplementation of the diet with organic  
235 or inorganic  $\text{Cr}^{3+}$  had no effect on feed intake or body weight gain in broilers reared under heat  
236 stress conditions. Furthermore, it was observed that chromium supplementation of the diet did  
237 not affect any performance parameters (body weight gain, feed intake or feed conversion ratio)  
238 in broilers (Ghazi et al., 2012). The differences among the outcomes recorded in various studies  
239 could be attributed to various factors such as the use of different forms of chromium, different  
240 environmental conditions (i.e., temperature, source of carbohydrate, diet level), the duration of  
241 treatment, the treatment dose, the response of the target birds to treatment, and the birds' health

242 status. Variable effects of chromium supplementation of the broiler diet on carcass  
243 characteristics have been reported in numerous studies (Anderson, 1987). The results of the  
244 current study confirm that variability. Cr<sup>3+</sup> supplementation at 0.10 mg/kg (group B)  
245 significantly increased the spleen index. However, Cr<sup>3+</sup> supplementation at 0.30 mg/kg  
246 significantly increased the thymus index in 21-day-old broilers. At 42 days of age, the birds in  
247 group D showed a significantly increased heart index compared to the other groups.  
248 Furthermore, the birds in groups C and F showed a significantly better thymus index compared  
249 to those of control B and E groups. However, the effects of chromium on the liver and the bursa  
250 of Fabricius were not significant. Similar variability has been reported in other studies (Rao et  
251 al., 2012; Hossain et al., 1998).

252 The results of the present study showed that dietary Cr<sup>3+</sup> supplementation boosted antibody titre  
253 against Newcastle disease and avian influenza. Treatment group C showed significantly higher  
254 antibody titre compared to the control and group E on day 7 post-immunization against  
255 Newcastle disease. Birds of groups C and D showed significantly higher antibody titres against  
256 Newcastle disease compared to the control and group E on day 21 post-immunization.  
257 Furthermore significantly higher titres than the control and group B on day 28 post-  
258 immunization. The antibody titre against H5 on day 21 after immunization was found to be  
259 significantly higher in the birds of group D compared to all other treatment groups, although it  
260 was similar to that of the control. Enhancement of the immune response of birds receiving  
261 dietary supplementation with chromium has also been reported by other authors (Sahin et al.,  
262 2005; Kheiri and Toghyani, 2009). The better immune response of the animals may be due to the  
263 antioxidant activity of chromium. This activity may decrease the production of corticosterone, a  
264 potent immunosuppressant that decreases leukocyte populations and obstructs lymphocyte

265 function and antibody production (Sahin et al., 2005; Lee et al., 2003). The results of the present  
266 study are consistent with those of Cao et al. (2004), who reported improved total antibody titres  
267 in broilers fed diets supplemented with chromium. Another reason for the better immune  
268 response may be that chromium modulated the immune response through its effects on  
269 cytokines. Cytokines are responsible for signalling between cells. Cytokines and their receptors  
270 affect the activities of other cells and play a major role in the regulation of the immune system  
271 (Davison, 2003; Callard et al., 1999).

272 It was essential to determine whether the health of the intestinal tract was improved by dietary  
273 supplementation with  $\text{Cr}^{3+}$  to promote nutrient utilization. It is recognized that enzymes in the  
274 digestive tract play vital roles in the digestion and absorption of nutrients. The birds in group C  
275 showed significantly greater crypt depth compared to the control and to the birds in group F at 21  
276 days of age. Groups C and F showed significantly increased WT in the jejunum compared to all  
277 other groups at 21 days of age. This may be due to the good health status of the birds and the use  
278 of proper management conditions, which can lead to better health of the digestive tract. These  
279 results contradict the findings of Makanjuola (2012) and Sandikci et al. (2004), who found a  
280 non-significant difference in crypt depth after chromium supplementation of broiler feed. In our  
281 study, dietary supplementation with different levels of  $\text{Cr}^{3+}$  probably led to an increase in the  
282 relative expression of genes encoding glucose and amino acid transporters in the intestinal tract  
283 at different stages of growth, and this is likely to have resulted in better absorption of nutrients  
284 and thus better utilization and improved growth performance of the birds.

285

## 286 **5. Conclusions**

287 Chromium has generally been used in the poultry industry to reduce heat stress. In the present  
288 study, dietary supplementation with Cr<sup>3+</sup> yielded reasonable results concerning immunity against  
289 diseases and intestinal morphology. In addition, Cr<sup>3+</sup> appears to have favourable effects on  
290 growth performance, especially on FCR, a finding that was verified by the higher expression of  
291 genes encoding nutrient transporters in different parts of the intestine. The concentrations of Cr<sup>3+</sup>  
292 (0.10, 0.15, 0.20, 0.25 or 0.30 mg/kg) used in the present study were found to be safe and can be  
293 used in the poultry industry to improve broiler health and performance. Supplementation of  
294 broiler feed with 0.15 mg/kg Cr<sup>3+</sup> was found to be most effective. Further studies conducted  
295 under different conditions are needed to assess the potential beneficial results of Cr<sup>3+</sup> in broiler  
296 production.

297

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305

### 306 **Conflict of interest**

307 The authors declare no conflicts of interest.

308

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- 409

410 **Figure legends**

411 **Figure 1. (a-c)** Growth performance of broilers after supplementation of the diet with different  
412 levels of chromium propionate. (a) (daily average feed intake g/bird at 1-21, 21-42 and 1-42 days  
413 of age); (b) (daily average weight gain g/bird at 1-21, 21-42 and 1-42 days of age); (c) (daily  
414 average FCR at 1-21, 21-42 and 1-42 days of age); 0 mg/kg: (basal diet with no chromium  
415 propionate supplementation; (b) 0.10 mg/kg: (basal diet+0.10 mg/kg chromium propionate  
416 supplementation); (c) 0.15 mg/kg: (basal diet+0.15 mg/kg chromium propionate  
417 supplementation); (d) 0.20 mg/kg: (basal diet+0.20 mg/kg chromium propionate  
418 supplementation); (e) 0.25 mg/kg: (basal diet+0.25 mg/kg chromium propionate  
419 supplementation); (b) 0.30 mg/kg: (basal diet+0.30 mg/kg chromium propionate  
420 supplementation). Means with different superscripts are significantly different ( $P < 0.05$ ). The  
421 data are presented as mean  $\pm$  SE.

422

423 **Figure 2. (a-f)** Duodenal villus histomorphometry of broilers at 42 days. (A) Control (basal diet  
424 with no supplementation); (B) (basal diet+chromium propionate 0.10 mg/kg; (C) (basal  
425 diet+chromium propionate 0.15 mg/kg); (D) (basal diet+chromium propionate 0.20 mg/kg); (E)  
426 (basal diet+chromium propionate 0.25 mg/kg); (F) (basal diet+chromium propionate 0.30  
427 mg/kg).

428

429 **Figure 3. (a-f)** Jejunal villus histomorphometry of broilers at 42 days. (A) Control (basal diet  
430 without any supplementation); (B) (basal diet+chromium propionate 0.10 mg/kg; (C) (basal  
431 diet+chromium propionate 0.15 mg/kg); (D) (basal diet+chromium propionate 0.20 mg/kg); (E)

432 (basal diet+chromium propionate 0.25 mg/kg); (F) (basal diet+chromium propionate 0.30  
433 mg/kg).

434

435 **Figure 4.** (a-f) Ileum villus histomorphometry of broilers at 42 days. (A) Control (basal diet  
436 without any supplementation); (B) (basal diet+chromium propionate 0.10 mg/kg; (C) (basal  
437 diet+chromium propionate 0.15 mg/kg); (D) (basal diet+chromium propionate 0.20 mg/kg); (E)  
438 (basal diet+chromium propionate 0.25 mg/kg); (F) (basal diet+chromium propionate 0.30  
439 mg/kg).

440

441 **Figure 5.** (a-f) mRNA expression of specific genes at 21 and 42 days of age in different regions  
442 of the intestine. (a) (duodenal mRNA expression at 21 days); (b) (duodenal mRNA expression at  
443 42 days); (c) (jejunal mRNA expression at 21 days); (d) (jejunal mRNA expression at 42 days;  
444 (e) (ileum mRNA expression at 21 days); (f) (ileum mRNA expression at 42 day) 0 mg/kg:  
445 (basal diet with no chromium propionate supplementation; (b) 0.10 mg/kg: (basal diet+ 0.10  
446 mg/kg chromium propionate supplementation); (c) 0.15 mg/kg: (basal diet+ 0.15 mg/kg  
447 chromium propionate supplementation); (d) 0.20 mg/kg: (basal diet+ 0.20 mg/kg chromium  
448 propionate supplementation); (e) 0.25 mg/kg: (basal diet+ 0.25 mg/kg chromium propionate  
449 supplementation); (b) 0.30 mg/kg: (basal diet+ 0.30 mg/kg chromium propionate  
450 supplementation). Means with different superscripts are significantly different ( $P < 0.05$ ). The  
451 data are presented as mean  $\pm$  SE.

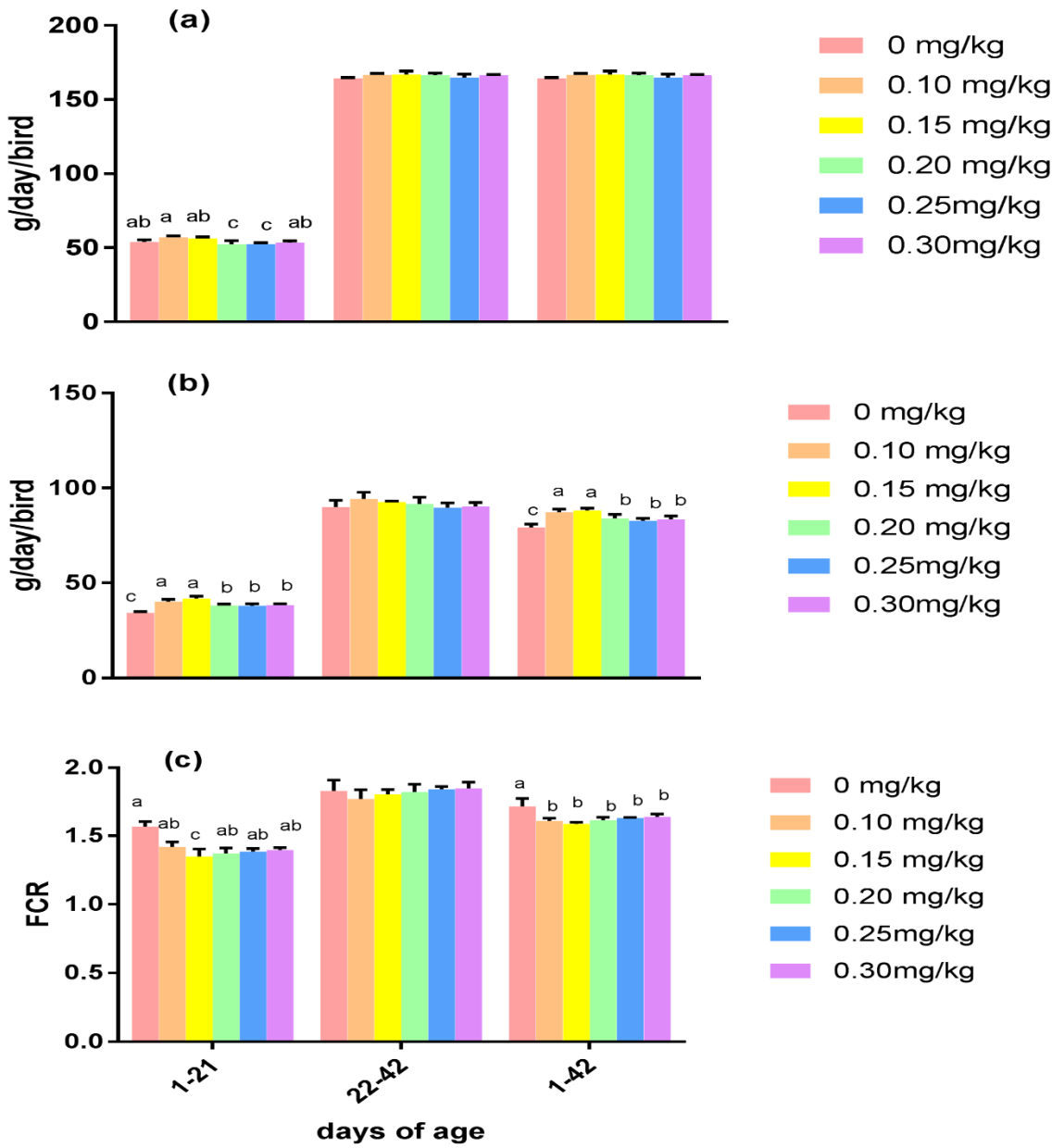
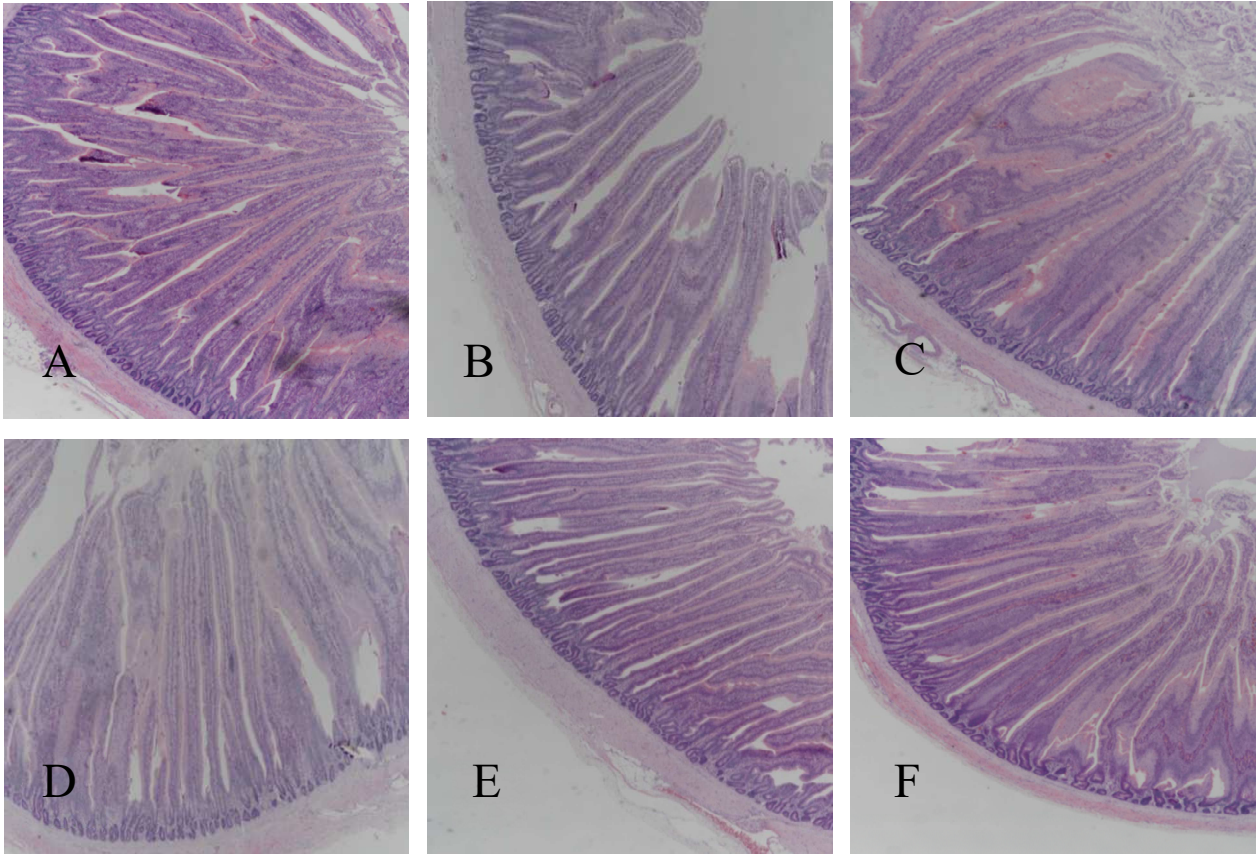
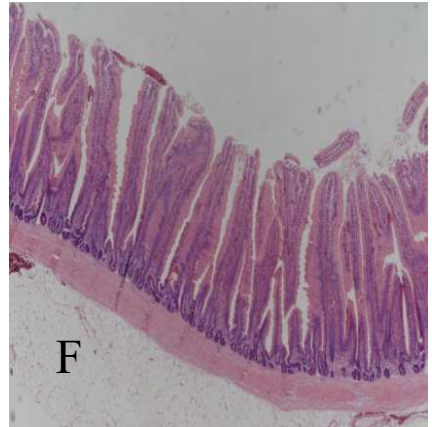
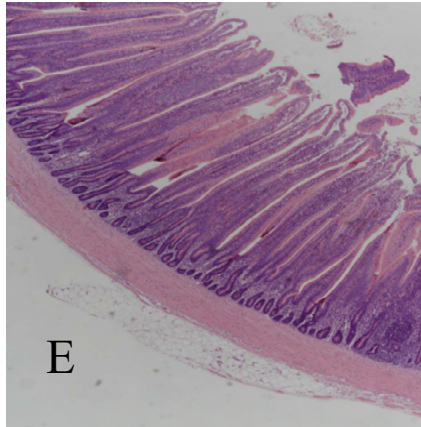
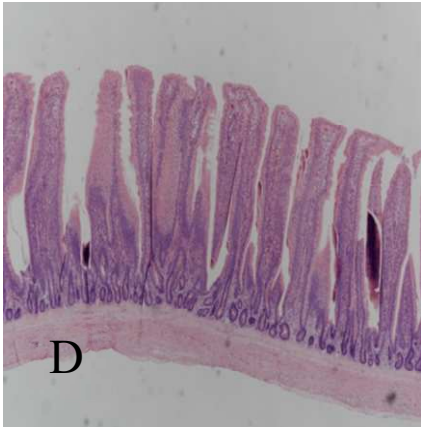
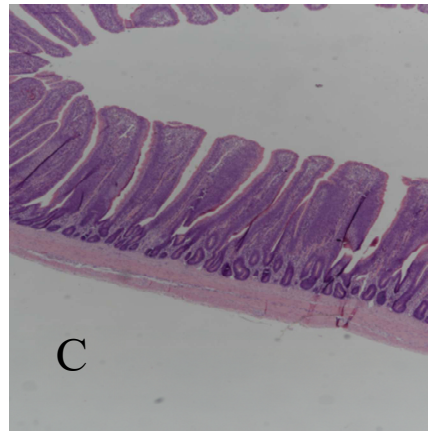
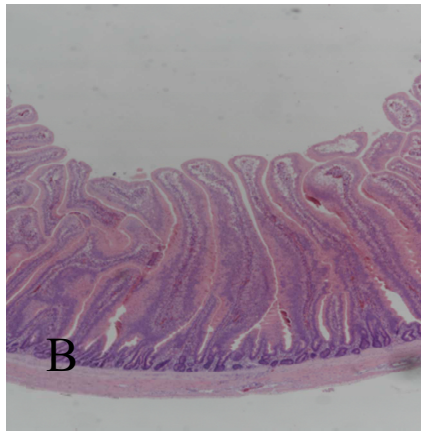


Figure 1.

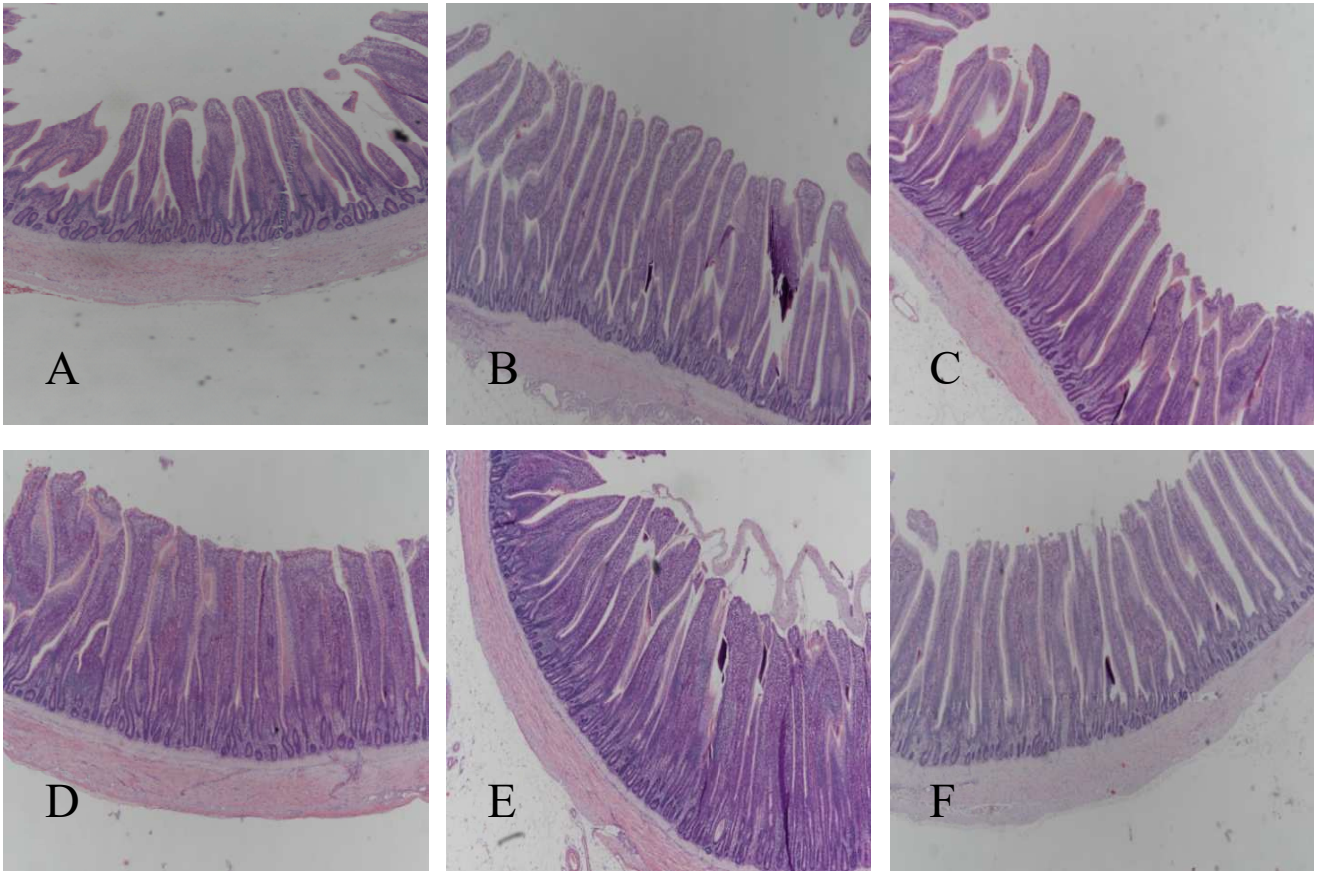


**Figure 2.**





**Figure 3.**



**Figure 4.**

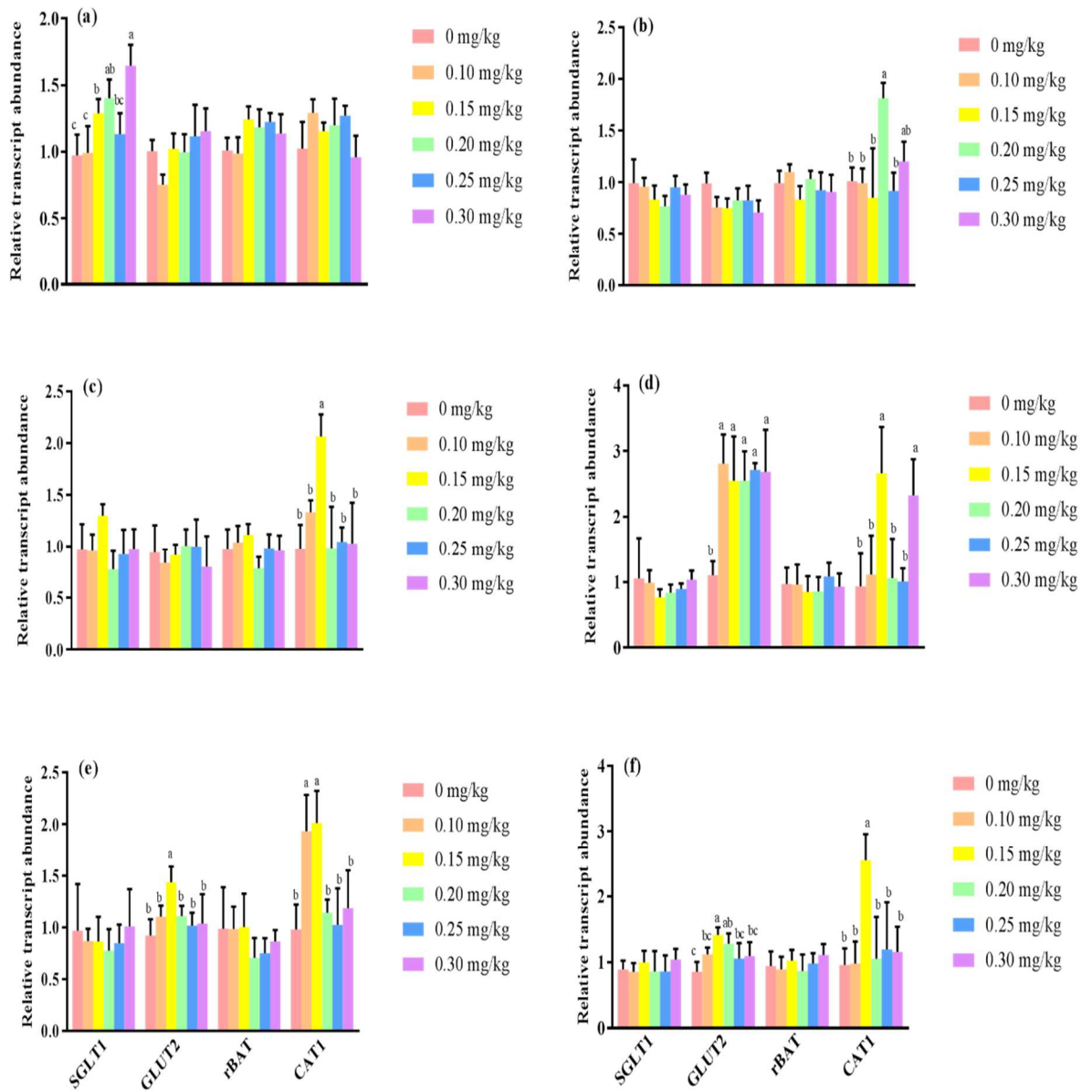


Figure 5.

**Table 1. Effect of dietary chromium propionate on the organ index of broilers**

Days	Parameters	Cr levels mg/kg						SEM	P Value
		0	0.10	0.15	0.20	0.25	0.30		
21	liver index	26.85	28.55	27.49	28.07	28.84	26.94	0.264	0.117
	Spleen index	0.79 <sup>bc</sup>	1.03 <sup>a</sup>	0.73 <sup>c</sup>	0.82 <sup>bc</sup>	0.92 <sup>ab</sup>	0.93 <sup>ab</sup>	0.029	0.021
	Heart index	5.26	5.74	5.44	5.52	5.68	5.59	0.057	0.213
	Thymus index	4.29 <sup>b</sup>	4.71 <sup>ab</sup>	4.71 <sup>ab</sup>	4.28 <sup>b</sup>	4.32 <sup>b</sup>	5.46 <sup>a</sup>	0.118	0.030
	Bursa index	1.66	1.63	1.78	1.84	2.02	1.82	0.051	0.312
	Liver index	22.48	21.36	22.97	22.15	22.16	24.19	0.386	0.448
42	Spleen index	1.41	1.14	1.19	1.25	1.34	1.15	0.039	0.313
	Heart index	4.06 <sup>b</sup>	4.06 <sup>b</sup>	4.60 <sup>a</sup>	3.59 <sup>c</sup>	4.15 <sup>b</sup>	4.07 <sup>b</sup>	0.075	0.002
	Thymus index	3.54 <sup>b</sup>	3.48 <sup>b</sup>	4.63 <sup>a</sup>	3.93 <sup>ab</sup>	3.23 <sup>b</sup>	4.43 <sup>a</sup>	0.128	0.002
	Bursa index	1.09	1.16	1.21	1.28	1.36	1.14	0.044	0.566

a, b, c Mean values within a row with different superscript letters were significantly different ( $P < 0.05$ ). Organ index =organ weight g/kg body weight. 0mg/kg: (basal diet, with no chromium propionate supplementation); (b) 0.10mg/kg: (basal diet+ 0.10 mg/kg chromium propionate supplementation); (c) 0.15mg/kg: (basal diet+ 0.15 mg/kg chromium propionate supplementation); (d) 0.20mg/kg: (basal diet+ 0.20 mg/kg chromium propionate supplementation); (e) 0.25mg/kg: (basal diet+ 0.25 mg/kg chromium propionate supplementation); (b) 0.30mg/kg: (basal diet+ 0.30 mg/kg chromium propionate supplementation). Means with different superscripts were significantly different ( $p < 0.05$ ). Data were presented as means  $\pm$  SE

**Table 2. Effect of dietary chromium propionate on serum antibody titer against Newcastle Disease and Avian Influenza in broilers**

Disease	Age	Cr levels (mg/kg)						SEM	P Value
		0	0.10	0.15	0.20	0.25	0.30		
Newcastle disease	Preimmune	6.75	6.75	6.00	6.00	7.50	7.00	1.42	0.867
	7 days after immunization	3.50 <sup>c</sup>	4.50 <sup>abc</sup>	5.00 <sup>a</sup>	4.75 <sup>ab</sup>	3.67 <sup>bc</sup>	4.00 <sup>abc</sup>	0.17	0.032
	14 days after immunization	6.75	6.50	6.25	6.25	5.75	5.75	0.18	0.575
	21 days after immunization	3.50 <sup>c</sup>	7.00 <sup>ab</sup>	7.25 <sup>a</sup>	8.00 <sup>a</sup>	5.00 <sup>bc</sup>	7.00 <sup>ab</sup>	0.397	0.001
	28 days after immunization	7.50 <sup>bc</sup>	6.25 <sup>c</sup>	9.25 <sup>a</sup>	9.00 <sup>a</sup>	8.00 <sup>ab</sup>	8.75 <sup>ab</sup>	0.265	0.001
H5 AI	21 days	8.50 <sup>ab</sup>	6.75 <sup>c</sup>	7.50 <sup>bc</sup>	8.75 <sup>a</sup>	7.25 <sup>c</sup>	7.00 <sup>c</sup>	0.20	0.002

a, b, c Mean values within a row with different superscript letters were significantly different ( $P < 0.05$ ). 0mg/kg: (basal diet, with no chromium propionate supplementation); (b) 0.10mg/kg: (basal diet+ 0.10 mg/kg chromium propionate supplementation); (c) 0.15mg/kg: (basal diet+ 0.15 mg/kg chromium propionate supplementation); (d) 0.20mg/kg: (basal diet+ 0.20 mg/kg chromium propionate supplementation); (e) 0.25mg/kg: (basal diet+ 0.25 mg/kg chromium propionate supplementation); (b) 0.30mg/kg: (basal diet+ 0.30 mg/kg chromium propionate supplementation). Means with different superscripts were significantly different ( $p < 0.05$ ). Data were presented as means  $\pm$  SE

**Table 3. Effects of dietary chromium propionate on intestinal morphology of duodenum in broilers**

Days	Parameters	Cr levels (mg/kg)						SEM	P Value
		0	0.10	0.15	0.20	0.25	0.30		
21	VH ( $\mu\text{m}$ )	3282.25	2878.00	3345.34	2965.79	3032.21	3198.09	68.273	0.298
	CD ( $\mu\text{m}$ )	161.96	177.83	171.84	193.96	164.38	165.42	4.357	0.287
	WT ( $\mu\text{m}$ )	460.33	396.34	435.42	425.17	411.42	414.96	9.297	0.482
	VH/CD	21.04	16.18	19.54	15.48	18.63	19.41	0.676	0.122
42	VH ( $\mu\text{m}$ )	3561.59	3123.96	3427.94	3645.17	3348.71	3176.62	99.952	0.635
	CD ( $\mu\text{m}$ )	25.957	23.73	17.90	29.70	14.44	20.59	4.750	0.475
	WT ( $\mu\text{m}$ )	515.21	504.96	526.95	494.55	512.63	476.63	12.099	0.915
	VH/CD	20.89	17.72	22.22	21.35	22.08	17.24	0.800	0.282

All values were non significant ( $P < 0.05$ ). 0mg/kg: (basal diet, with no chromium propionate supplementation); (b) 0.10mg/kg: (basal diet+ 0.10 mg/kg chromium propionate supplementation); (c) 0.15mg/kg: (basal diet+ 0.15 mg/kg chromium propionate supplementation); (d) 0.20mg/kg: (basal diet+ 0.20 mg/kg chromium propionate supplementation); (e) 0.25mg/kg: (basal diet+ 0.25 mg/kg chromium propionate supplementation); (b) 0.30mg/kg: (basal diet+ 0.30 mg/kg chromium propionate supplementation). Means with different superscripts were significantly different ( $p < 0.05$ ). Data were presented as means  $\pm$  SE; VH (Villus height), CD (Crypt depth), WT (Wall thickness)

**Table 4. Effect of dietary chromium propionate on intestinal morphology of jejunum in broilers**

Days	Parameters	Cr levels (mg/kg)						SEM	P Value
		0	0.10	0.15	0.20	0.25	0.30		
21	VH ( $\mu\text{m}$ )	2591.63	2117.16	2237.79	2111.59	2098.63	2451.00	82.222	0.405
	CD ( $\mu\text{m}$ )	159.33 <sup>bc</sup>	190.67 <sup>ab</sup>	197.25 <sup>a</sup>	193.25 <sup>ab</sup>	176.09 <sup>abc</sup>	143.25 <sup>c</sup>	5.843	0.023
	WT ( $\mu\text{m}$ )	407.79 <sup>b</sup>	391.71 <sup>b</sup>	522.88 <sup>a</sup>	395.09 <sup>b</sup>	423.54 <sup>b</sup>	446.88 <sup>ab</sup>	13.599	0.029
	VH/CD	16.82 <sup>a</sup>	11.07 <sup>b</sup>	11.60 <sup>b</sup>	11.16 <sup>b</sup>	11.91 <sup>b</sup>	17.16 <sup>a</sup>	0.760	0.014
42	VH ( $\mu\text{m}$ )	2511.17	2550.21	2144.67	2206.88	2228.13	2126.50	83.058	0.578
	CD ( $\mu\text{m}$ )	172.17	178.96	192.38	208.84	184.33	170.75	6.604	0.604
	WT ( $\mu\text{m}$ )	481.28	487.25	465.29	494.33	448.87	453.96	13.338	0.919
	VH/CD	15.15	14.54	11.58	11.04	12.19	12.52	0.666	0.478

a, b, c Mean values within a row with different superscript letters were significantly different ( $P < 0.05$ ). 0mg/kg: (basal diet, with no chromium propionate supplementation); (b) 0.10mg/kg: (basal diet+ 0.10 mg/kg chromium propionate supplementation); (c) 0.15mg/kg: (basal diet+ 0.15 mg/kg chromium propionate supplementation); (d) 0.20mg/kg: (basal diet+ 0.20 mg/kg chromium propionate supplementation); (e) 0.25mg/kg: (basal diet+ 0.25 mg/kg chromium propionate supplementation); (b) 0.30mg/kg: (basal diet+ 0.30 mg/kg chromium propionate supplementation). Means with different superscripts were significantly different ( $p < 0.05$ ). Data were presented as means  $\pm$  SE; VH (Villus height), CD (Crypt depth), WT (Wall thickness)

**Table 5. Effect of dietary chromium propionate on intestinal morphology of ileum in broilers**

Days	Parameters	Cr levels (mg/kg)						SEM	P Value
		0	0.10	0.15	0.20	0.25	0.30		
21	VH ( $\mu\text{m}$ )	1678.17	1424.21	1502.25	1588.79	1599.56	1510.54	58.405	0.880
	CD ( $\mu\text{m}$ )	189.75	162.92	183.62	187.04	152.33	183.04	4.545	0.116
	WT ( $\mu\text{m}$ )	530.96	490.75	488.71	512.50	469.94	448.17	16.297	0.779
	VH/CD	8.94	8.76	8.15	8.46	10.70	8.26	0.387	0.572
42	VH ( $\mu\text{m}$ )	1560.50	1776.75	1741.04	1730.17	1832.63	1793.56	61.922	0.945
	CD ( $\mu\text{m}$ )	192.92	162.58	178.84	166.06	182.00	166.83	6.096	0.811
	WT ( $\mu\text{m}$ )	621.00	483.00	548.71	541.50	534.96	527.94	25.735	0.886
	VH/CD	8.01	10.91	9.76	10.58	10.31	10.87	0.376	0.447

All values were non significantly ( $P < 0.05$ ). 0mg/kg: (basal diet, with no chromium propionate supplementation); (b) 0.10mg/kg: (basal diet+ 0.10 mg/kg chromium propionate supplementation); (c) 0.15mg/kg: (basal diet+ 0.15 mg/kg chromium propionate supplementation); (d) 0.20mg/kg: (basal diet+ 0.20 mg/kg chromium propionate supplementation); (e) 0.25mg/kg: (basal diet+ 0.25 mg/kg chromium propionate supplementation); (b) 0.30mg/kg: (basal diet+ 0.30 mg/kg chromium propionate supplementation). Means with different superscripts were significantly different ( $p < 0.05$ ). Data were presented as means  $\pm$  SE; VH (Villus height), CD (Crypt depth), WT (Wall thickness)



**Table 6. Feed composition offered to broilers during experiment**

Ingredients	Starter (%) (1-21days)	Finisher (%) (22-42 days)
Corn	52.24	55.69
Soybean meal	32.17	30.1
Cotton seed meal	2	3
Corn Gluten 60%	4	4
Rapeseed meal	3	3
Calcium	2.5	1.5
Phosphate (powder)	1.83	0.12
Lysine sulphate 70%	0.77	1.06
Salt	0.3	0.36
DL-methionine	0.28	0.30
*Premix	0.3	0.3
Threonine	0.17	0.24
Zeolite powder	0.17	0.15
Choline chloride (50%)	0.15	0.1
Tryptophan	0.12	0.08
Total	100	100
<b>Chemical Composition</b>		
Crude Protein % (CP)	21.2	19
Calcium %	0.96	0.85
Phosphorous %	0.70	0.58
Lysine %	1.294	1.272
Methionine %	0.603	0.651
Metabolic Energy kcal/kg	2930	3050

\*the premix added in one kg of the diet: Vit. A, 250000 I.U; Vit. D3, 50000 I.U.; Vit. E, 600 I.U; Vit. K3, 40 mg; Vit. B1, 40 mg; Vit. B2, 120mg; B5, 240 mg; Vit.B12, 0.50 mg; Niacin, 1 g; Biotin, 0.65 mg; Folic acid, 25 mg; Zn, 75 mg; Mn, 80 mg; Fe, 80 mg; Cu, 10 mg; I, 0.40 mg; Se, 0.30 mg.

**Table 7. The sequences of primers used in quantitative real-time PCR Target gene Primer Sequence (5'–3')**

Target Gene	Primer	Sequence	GenBank Accession No.
Beta-Actin	Forward	ATTGTCCACCGCAAATGCTTC	NM_205518.1
	Reverse	AAATAAAGCCATGCCAATCTCGTC	
SGLT1	Forward	AGCATTTTCAGCATGGTGTGTCTTC	XM_415247
	Reverse	GATGCTCCTATCTCAGGGCAGTTC	
GLUT2	Forward	CACACTATGGGCGCATGCT	XM_205209.1
	Reverse	ATTGTCCCTGGAGGTGTTGGTG	
rBAT	Forward	CCCGCCGTTCAACAAGAG	XM_426125
	Reverse	AATTAAATCCATCGACTCCTTTGC	
CAT1	Forward	CAAGAGGAAAACCTCCAGTAATTGCA	NC_003424.3
	Reverse	AAGTCGAAGAGGAAGGCCATAA	

## Chromium propionate $\text{Cr}^{3+}$



Broiler chickens

