Influence of early feeding with different diet composition on performance and intestinal morphology of layer-type chicks during the brooding period

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ABSTRACT. The study was conducted to examine the effects of early feeding with diets of different composition or fasting for 72 h on performance, intestinal organs development and intestinal histomorphology of layer-type chicks during the brooding period. In total, 400 chicks (50% males, 50% females) were randomly allocated to 4 treatments with 4 replications of 25 chicks each. The dietary treatments during the first 72 h of life were: T1 (control) – fasting, T2 – a starter layer diet containing crude protein (CP) 20% and metabolizable energy (ME) 11.84 MJ/kg, T3 – a diet containing 20% CP and 11.81 MJ/kg ME (3% molasses in its composition), and T4 – a starter broiler diet (23% CP and 12.68 MJ/kg ME). For the whole 42-day experimental period, it was indicated that birds fed T4 and T3 diets had higher body weight gain and better feed conversion ratio than fasted ones. Chicks fed T4 diet had the highest intestinal weight and length at day 14 of age. Whereas, birds from T3 group had increased gizzard weight than other groups both at days 14 and 28 of age. At day 14 of age, increased duodenal villus height and width and smaller crypt depth were recorded in T4 group in comparison to fasted and other groups of animals. Longer villi and smaller crypt depth of jejunum were found in T4 and T3 chicks than in fasted ones. Longer ileal villi was observed in T4 birds at day 14 of age. The obtained results suggest that early feeding with a diet rich in CP or containing molasses addition has beneficial effects on the production performance and intestinal development of layer-type chicks.

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Introduction

In the current poultry industry, birds often do not have free access to feed and water during the first 48–72 h post-hatch due to differences in the time of hatching and management practices. Delayed access to feed and water post-hatch has been reported to negatively influence yolk utilization, survivability, gastrointestinal development, carcass yield and immunological development (e.g., Yang et al., 2009; Pretorius, 2011; Shinde et al., 2015; Sarlak et al., 2016). Saki (2005) reported a linear relationship between the weight of the first week of rearing and the slaughter weight in broiler chickens. Therefore, early feeding of post-hatch chicks could greatly influence the growth performance of broiler chickens (Yang et al., 2009). Birds that have early access to a high amino acid diet, even in the presence of sub-
clinical necrotic enteritis, were shown to have better performance and immune response (Keerqin et al., 2017).

The yolk sac of the hatching chicks is a good source of fat and protein, but it has low carbohydrates content (Uni and Ferket, 2004). Investigation of chicks close to hatch indicates that yolk during the first 48 h post-hatch contributes to the development of the small intestine (Noy and Sklan, 1999). Newly hatchlings are less efficient in utilizing nutrients, and diets containing easily digestible nutrients can be beneficial for them (Pretorius, 2011). Inclusion of highly digestible raw materials in the pre-starter diets, such as soy protein, glucose-based products and simple sugars, was shown to have positive impacts on the production performance of broiler chickens (Batal and Parsons, 2004; Longo et al., 2005). Recently, Sarlak et al. (2016) observed that feeding a maize-soybean meal (2850 kcal/kg ME and 22.73% CP) as a starter diet for newly hatched chicks has a beneficial effect on their performance, immune status and morphological development of the intestine.

Suitable diet composition and optimal feed formulation specified during the first 72 h of layer-type chicks life are limited. Inclusion of highly digestible proteins and carbohydrates in the pre-starter diet could be used as an investment in poultry production to minimize the need for gluconeogenesis in order to maintain body reserves. Therefore, the current study was designed to investigate the effects of early feeding with different diet composition and fasting during the first 72 h of post-hatching on growth performance, and intestinal development and histomorphology of layer-type chicks during the brooding period.

Material and methods

Hatching eggs, chicks and housing

All the procedures used in the current study have been approved by the Ethical Commitee of Faculty of Agriculture, Menoufia University (Egypt). Eggs from Norfa laying breeder flock (crossing of exotic and local egg breeds, White Leghorn × Fayoumi × White Baladi (Kolstad and Abdou, 2000) at the age of 72 weeks) weighing between 40 and 45 g were taken and set for incubation at an egg shell temperature of 37.8 °C and relative humidity about 50% in a commercial hatchery (Poultry Research Farm, Faculty of Agriculture, Menoufia University, Egypt). On day 18 of the incubation period, eggs were transferred to the hatchery. Hatching trays were checked every 4 h, and chicks that had hatched were weighed and wing-banded, and the hatching time was reported. A group of 400 hatched chicks (50% males, 50% females) was used as experimental birds. Hatching trays were divided with partitions and 4 different treatments were carried out. All chicks were transferred from the hatchery after 72 h post-hatching and placed in the floor pens, bedded with clean wood shavings. The light regimen was continuous during the first week and then decreased to 23 h of light thereafter. Room temperature was kept at 33 °C for the first week and 30 °C for the second week, which was further gradually reduced to 22 °C till the end of the experiment. All experimental chicks were reared under the same standard management practices and routine vaccination.

Experimental diets

A completely randomized design with 4 treatments and 4 replications per treatment with 25 birds per replicate was used in the study. Four dietary treatments: T1 (control) – fasting, T2 – a starter layer diet containing crude protein (CP) 20% and metabolizable energy (ME) 11.84 MJ/kg, T3 – a diet containing 20% CP and 11.81 MJ/kg ME (3% molasses in its composition), and T4 – a starter broiler diet (23% CP and 12.68 MJ/kg ME were considered during the first 72 h of chicks life. All chicks 72 h immediately post-hatching (after fasting or feeding experimental diets) were fed layer starter mash diet (20% CP and 11.84 MJ/kg ME, T2) ad libitum for a period of 42 days post-hatch. The recommendation tables of National Research Council (NRC, 1994) for the nutrient requirements of white-egg breeders were utilized to calculate the nutrient requirements for Norfa layer chicks (Abdel Rahman and Attia, 2001; Zanaty, 2006). The composition of the experimental diet was based on NRC (1994) tabulated values for feedstuffs. The compositions, nutrient concentrations and the proximate chemical analysis, namely dry matter (DM), CP, ether extract (EE) as well as crude fibre (CF) and ash (AOAC International, 2005) of the diets are presented in Table 1.

Sampling, measurements and methods of analysis

Body weights of birds were determined at days 1, 14, 28 and 42 of age. Feed intake and weight gain were recorded in the different experimental periods and feed conversion ratio (feed intake/weight gain) was calculated accordingly. Mortality was recorded when it occurred. At days 14 and 28 of age,
two chicks per replicate, close to the average group mean weight, were selected, weighted and sacrificed by cervical dislocation. The gastrointestinal tract from the proventriculus to the end of the intestine was excised. The small intestine (after removal of contents), empty gizzard and liver were weighted and excised from duodenum, jejunum, and ileum at days 14 and 28 of age. Segments referred to the midpoint (from gizzard to pancreo-biliary duct), jejunum (the midpoint between the entry of the common bile duct and the Meckel’s diverticulum) and ileum (from the Meckel’s diverticulum to ileocecal junction). Particular segments were gently flushed and rinsed with 0.9% physiological saline and then fixed in 4% neutral-buffered formalin solution for histological study.

Samples of the small intestine were transferred from formaldehyde after dehydration through passing tissue by sequences of alcohol solutions, and then were cleared by xylene and embedded in paraffin. All samples were sectioned at 5-µm thickness by using a rotary microtome. Sections were sequentially transferred to glass slides and stained with hematoxylin and eosin. After glass slides being dried, sections were examined using a light microscope. Morphometric measurements were performed on the selected 9 villi from each sample. The height of intestinal villi was measured from the tip to the base of villi at the opening crypt, and the villus width was measured at its midpoint (Geyra et al., 2001). The intestinal crypt depth was measured from the base of the villi to submucosa, and the muscular thickness from the submucosa to the external layer of the intestine (Geyra et al., 2001).

### Statistical analysis

Experimental data were subjected to the analysis of variance (ANOVA) using IBM SPSS Statistics 22 statistical package (SPSS Inc., Chicago, IL, USA) as a completely randomized design. Significant differences among the treatments were determined using Tukey’s test at $P < 0.05$.

### Results

#### Growth performance

The body weights of layer chicks were different during the whole experimental period as a result of fasting and compositions of different early diets (Table 2). Birds offered diets with different compositions had significantly ($P < 0.05$) higher body weight 72 h after hatch, at weeks 2 (except T2 group), 4 (except T2 and T3 groups) and 6 (except T2 group) of age in comparison to those deprived of feed and water (T1). Data revealed that at week 6 of age birds fed T4, and those fed T3 diets had higher ($P = 0.04$) body weight than those fed starter layer diet (T2) or fasted for the first 72 h post-hatching. Chicks fed T4 and T3 diets during 72 h post-hatch had also significantly higher body weight gain ($P = 0.03$) in comparison to control (T1) and T2 groups during the whole period from hatch to day 42 of life.
Feed intake during the entire experimental period was significantly \((P < 0.001)\) influenced by the composition of early diet (level of protein and energy density). Chicks offered T3 treatment were characterized by significantly higher feed intake \((P < 0.05)\) than those fed T4 and T2 diets during the first 72 h of life \((5.73 \text{ vs} 4.98 \text{ and} 4.90 \text{ g/d} \pm 0.12, \text{ respectively})\). Chicks fed T4 diet, followed by those fed T3 showed lower feed intake \((P = 0.001)\) than those fed T2 diet and fasted ones \((T1)\) during the entire experimental period from the hatch to day 42 \((Table 2)\). Early feeding during the first 72 h of life \((T4, \text{ followed by} \ T3 \text{ and} \ T2)\) was shown to significantly improve FCR of chicks during days 0–14 \((P = 0.002)\), 15–28 \((P = 0.001)\) and 29–42 \((P = 0.001)\) as compared to fasting ones.

### Post-hatch intestinal organ development

Data of post-hatch intestinal organ development, such as the small intestine weight and length, and liver and gizzard weights at days 14 and 28 of age are presented in Table 3. Intestinal weight \((P = 0.004)\) and length \((P = 0.001)\) were significantly higher in the T4 group than in fasted and other treatment groups at day 14 of age. Whereas, T2 and T3 chicks had lower intestinal relative weight and length \((P < 0.05)\) in comparison to the control group \((T1)\) at day 28 of age. The relative weight of gizzard was significantly higher in T3 chicks than in the other animals at days 14 \((P = 0.002)\) and 28 \((P < 0.05)\) of age. Chicks fed T4 diet immediately after hatch had higher relative weight of the liver \((P < 0.05)\) at day 14 of age than those from fasted and T2 groups. There was no significant difference in the relative weight of the liver at day 28 of life among the groups.

### Intestinal development and morphology

The average villus height, villus width, crypt depth and muscular thickness of duodenum, jejunum and ileum of birds measured at days 14 and 28 of age are presented in Table 4. In the duodenum, birds fed T4 diet for 72 h after hatching had significantly higher villus height and smaller crypt depth \((P < 0.001)\) at day 14 of age than those fasted or fed T2 and T3 diets post-hatch. However, there was no significant difference in villus height of the duodenum at day 28 of age between the control-fasted and other treatment groups. The highest villus width of the duodenum in relation to other groups \((P = 0.002)\) was observed at day 14 of age in chicks fed T4 diet after hatching compared to fasted and T2 ones. While, there was no difference in duodenal villus width between fasted and T2 birds or between T3 and T4 birds. At day 28 of age, birds from T2 group had narrower duodenal villus width \((P = 0.001)\) than other treatments. Smaller duodenal

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**Table 2. Effect of early feeding with diets of different composition on the production performance of chicks during the brooding period**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Early feeding treatments(^1)</th>
<th>SEM(^2)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>after 72 h post-hatch</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>day 14</td>
<td>31.0abc</td>
<td>33.2a</td>
<td>33.5ab</td>
</tr>
<tr>
<td>day 28</td>
<td>80.5abc</td>
<td>83.8a</td>
<td>85.9a</td>
</tr>
<tr>
<td>day 42</td>
<td>147ab</td>
<td>153ab</td>
<td>155ab</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>hatch–14 days</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>14–28 days</td>
<td>49.5abc</td>
<td>60.6a</td>
<td>52.4a</td>
</tr>
<tr>
<td>28–42 days</td>
<td>110abc</td>
<td>114ab</td>
<td>127a</td>
</tr>
<tr>
<td>Feed intake, g/chick</td>
<td>hatch–14 days</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>14–28 days</td>
<td>151a</td>
<td>150a</td>
<td>145a</td>
</tr>
<tr>
<td>28–42 days</td>
<td>231a</td>
<td>226a</td>
<td>221a</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>hatch–14 days</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>14–28 days</td>
<td>3.05abc</td>
<td>2.98ab</td>
<td>2.75a</td>
</tr>
<tr>
<td>28–42 days</td>
<td>3.49abc</td>
<td>3.27ab</td>
<td>3.18a</td>
</tr>
</tbody>
</table>
| Intestinal development and morphology

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**Table 3. Effect of early feeding with diets of different composition on the intestinal weight, intestinal length and weight of digestive organs of chicks during the brooding period, % of live BW**

<table>
<thead>
<tr>
<th>Indices</th>
<th>Early feeding treatments(^1)</th>
<th>SEM(^2)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At day 14 of age</td>
<td>intestinal weight</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>6.95a</td>
<td>7.39a</td>
<td>7.24a</td>
<td>9.25a</td>
</tr>
<tr>
<td>At day 28 of age</td>
<td>intestinal weight</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>11.30a</td>
<td>10.96a</td>
<td>10.80a</td>
<td>10.90a</td>
</tr>
<tr>
<td>Intestinal length</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>85.5a</td>
<td>74.5a</td>
<td>77.0a</td>
<td>83.0a</td>
</tr>
<tr>
<td>Gizzard weight</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>8.10a</td>
<td>8.09a</td>
<td>9.30a</td>
<td>7.92a</td>
</tr>
</tbody>
</table>

\(^1\) – values in the same row with different superscripts are significantly different at \(P < 0.05\)

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\(^2\) – see Table 2; – values in the same row with different superscripts are significantly different at \(P < 0.05\)
Early feeding treatments

<table>
<thead>
<tr>
<th>Indices</th>
<th>Early feeding treatments</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>At day 14 of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| villi height   | 1105   | 1134 | 1229 | 1353 | 32.9          | <0.001
| villi width    | 127    | 170  | 202  | 250  | 17.2          | 0.002
| crypt depth    | 252    | 272  | 244  | 145  | 15.7          | 0.001
| muscular thickness | 150  | 216  | 244  | 250  | 17.6          | 0.002
| Jejunum        |          |      |      |      |               |
| villi height   | 737    | 942  | 1038 | 1093 | 24.5          | <0.001
| villi width    | 175    | 253  | 263  | 285  | 12.3          | 0.001
| crypt depth    | 276    | 294  | 205  | 177  | 16.9          | <0.001
| muscular thickness | 169  | 160  | 191  | 179  | 11.8          | 0.13
| Ileum          |          |      |      |      |               |
| villi height   | 537    | 738  | 742  | 893  | 32.7          | 0.004
| villi width    | 185    | 165  | 153  | 163  | 15.5          | 0.29
| crypt depth    | 193    | 204  | 215  | 216  | 18.5          | 0.58
| muscular thickness | 164  | 175  | 186  | 174  | 8.4           | 0.15
| At day 28 of age |          |      |      |      |               |
| Duodenum       |          |      |      |      |               |
| villi height   | 1680   | 1613 | 1591 | 1611 | 32.7          | 0.11
| villi width    | 275    | 129  | 229  | 258  | 20.4          | 0.001
| crypt depth    | 272    | 255  | 240  | 218  | 14.7          | 0.03
| muscular thickness | 262  | 240  | 256  | 266  | 10.0          | 0.12
| Jejunum        |          |      |      |      |               |
| villi height   | 1304   | 1336 | 1340 | 1363 | 30.1          | 0.34
| villi width    | 252    | 232  | 255  | 214  | 14.8          | 0.08
| crypt depth    | 220    | 198  | 223  | 199  | 10.7          | 0.09
| muscular thickness | 285  | 261  | 240  | 251  | 9.4           | 0.008
| Ileum          |          |      |      |      |               |
| villi height   | 894    | 936  | 904  | 963  | 27.3          | 0.11
| villi width    | 248    | 229  | 251  | 220  | 16.9          | 0.28
| crypt depth    | 209    | 200  | 171  | 190  | 12.2          | 0.07
| muscular thickness | 262  | 258  | 237  | 248  | 12.5          | 0.26

\( ^{12} \) see Table 2; \( ^{a} \) values in the same row with different superscripts are significantly different at \( P < 0.05 \)

Discussion

Early access to different forms of feed, either liquid or solid, has been reported to enhance the growth performance of birds in comparison to those with delayed access to feed (Batal and Parsons, 2004; Longo et al., 2005; Saki, 2005; Yang et al., 2009). Under commercial conditions, the absence of feed within the hatchery for 24–48 h has been reported to induce a linear reduction in poultry body weight at 0.17 g/h (Sklan et al., 2000). This loss in body weight post-hatch is mainly attributed to the metabolic changes, yolk consumption and dehydration occurring in the hatchery during the holding period.

In the present study, the lower body weights of fasted chicks after the holding time (72-h post-hatch) and at the end of the experiment (day 42 of age) were in agreement with previous studies (Abed et al., 2011; Sanca et al., 2014; Sarlak et al., 2016) which reported that feed deprivation during the first 48 h after hatching had adverse effects on the growth performance of broilers. Decreased body weights of fasted chicks could be mainly attributed to a decrease in feed intake and FCR (Abed et al., 2011; Shinde et al., 2015; Sarlak et al., 2016). However, in the present study the lower feed intake, greater body weight gain and better FCR during 0–42 days of age were observed in birds fed T4, followed by those fed T3 and T2 diets for 72-h post-hatch. The results of the current study suggest that feed and water deprivation during the initial 72 h of life has adverse effect on body weight gain, which could be contributed to a decrease in FCR rather than feed consumption. Other researchers (e.g., Gonzales et al., 2003; Tabedian et al., 2010) reported lower feed intake and higher FCR at days 21 and 42 of age in broilers deprived of feed and water for 24- or 48-h post-hatch. On the other hand, Abed et al. (2011) observed a lower feed intake and FCR in broilers deprived of feed and water for 48-h post-hatch in comparison to control at day 11 of age. The reason for this inconsistency between the studies is unclear.
Inclusion of 3% molasses into diet during early feeding resulted in an increase in body weight gain and better FCR than in animals that were feed-deprived. Our findings are consistent with the results of other researchers (Batal and Parsons, 2004; Longo et al., 2005), who stated that the inclusion of sugars in the pre-starter diet led to a significant increase in broiler performance. Recently, Sarlak et al. (2016) observed that birds fed maize gluten-dextrose diet for 5 days after hatching had lower feed intake and body weight gain than those fasted for 24 h post-hatch or those fed maize-soybean meal diet immediately after hatching. In the study by Sarlak et al. (2016), the lowered feed intake and body weight gain may be attributed to the imbalanced amino acid structure of the used maize gluten meal.

In the present study, the relative intestinal weight and length at day 14 of age was higher in T4 chicks than in fasted and other treatment groups. It was suggested by Sklan and Noy (2003) that chicks might have high protein requirements post-hatching for specific tissues development such as the small intestine (Sklan, 2001). Previous studies (e.g., Mikec et al., 2006; Abed et al., 2011; Sarca et al., 2014) demonstrated that birds deprived of feed post-hatch had lower intestinal weight and length in comparison to those fed typical maize-soybean meal diet. The highest weight and length of the small intestine in the fasted group at day 28 of age in the current study was rather unexpected. Also, Sarlak et al. (2016) observed that the relative weight and length of the small intestine at day 42 of age was higher in broilers fasted for 48 h post-hatching. It seems that the higher intestinal weight and length in fasted chicks could be an adaptive compensatory mechanism to allow complete recovery of the growth.

The relative weight of gizzard was higher in T3 group in comparison to the other groups at days 14 and 28 of age. These findings are in agreement with those reported by El-Husseiny et al. (2008) who observed that feed deprivation during the first 48 h post-hatch affected intestinal organ development. The current findings suggested that early access to feed rich in CP content or containing molasses stimulates the growth of the small intestine and gizzard. It has been demonstrated that the nutrients found in the yolk were used for intestinal development (Noy and Sklan, 1999). This may clarify why early feeding enhances intestine and gizzard development in comparison to feed deprived chicks. On the contrary, Shinde et al. (2015) and Sarlak et al. (2016) reported that there was no significant difference in the relative weight of gizzard between fasted and early fed birds. The difference between the current study and previous studies could contribute to the different composition of early diet.

Feed deprivation and early feeding with different diet composition affected the structure of the small intestine in layer-type chicks. The histomorphological examination of the small intestine segments at day 14 of age revealed that early feeding a diet containing 23% CP and 12.68 MJ/kg ME (T4) increased duodenal villi height and width, but decreased crypt depth in comparison to treatments (including fasting animals). Furthermore, feed deprived chicks had lower jejunal and ileal villus height and greater jejunal crypt depth in comparison to T3 and T4 groups. The villi height and crypt depth of the absorptive small intestinal epithelium play key roles in the digestion, absorption and assimilation of nutrients (Wang and Peng, 2008). The crypt depth is one of the healthiest and functional histomorphometric parameters of the intestinal status in chicks (Samanya and Yamauchi, 2002). Crypt depth can be used as an indicator of the strength and renewal processes of the intestinal epithelium (Samanya and Yamauchi, 2002), indicating that larger crypts have rapid tissue turnover and a high demand for new epithelium (Xu et al., 2003). In the current study, decreased crypt depth in T4 group may indicate an efficient tissue turnover and good gut condition (Yegani and Korver, 2008). The adverse effects of feed deprivation immediately post-hatching on the small intestine villus height, width and crypt depth were also observed in previous studies (Sarca et al., 2014; Sarlak et al., 2016). Shorter villi and deeper crypts may lead to reduced nutrient absorption, increased digestive secretion and diarrhea, which in turn diminished growth performance (Yegani and Korver, 2008). Additionally, the tunica muscularis was thicker in the duodenum of early fed birds in comparison to fasted ones, which may enhance the contact between the mucosa and intestinal content (de Verdal et al., 2010). Therefore, the negative effects of feed deprivation post-hatching on the growth performance of chicks may be correlated with the morphological changes in the development of the small intestine, particularly duodenum and jejenum.

Conclusions

Feed withdrawal during the first 72 h immediately after hatching had negative effects on the performance traits of layer-type chicks during the brooding period. Early feeding of a diet containing 23% of crude protein (CP) and 12.68 MJ/kg of metabolizable energy (ME) followed by that containing 20% of CP
Fasting vs early feeding influence on chicks performance and intestinal morphology

and 11.81 MJ/kg of ME with 3% molasses in its nutrient composition improved performance traits in comparison to feed deprivation. The adverse effects of fasting on the growth performance of layer-type chicks were correlated with the morphological changes in the small intestine, particularly duodenum and jejunum. The current study suggested that early feeding immediately after hatching has beneficial effects on the growth performance and small intestine development of layer-type chicks during the brooding period. Early feeding vs feed deprivation and their relations with the secretion of digestive enzymes, histological examination of immune organs and the expression of nutrient transporter related genes in layer-type chicks need to be further clarified in future studies.

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