The effect of different early feeding regimens involving a hydrated nutritious gel on productive performance, immune variables, and intestinal morphology of broiler chickens

Alireza Hesabi Nameghi\textsuperscript{a}, Ali Nasari Nejad\textsuperscript{b}, Marzieh Afkhami\textsuperscript{b,c}, Farhad Khaligh\textsuperscript{b,c} and Omid Behrouzi Nasab\textsuperscript{b,d}

\textsuperscript{a}Department of Animal Science Research, Agricultural Research, Education and Extension Organization, (AREEO), Mashhad, Iran; \textsuperscript{b}Research and Development Department of Tehran Toyur Sabz Andishan Bartar Company, Mashhad, Iran; \textsuperscript{c}Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; \textsuperscript{d}Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

\textbf{ABSTRACT}

The present research aimed to evaluate the effect of early feeding (EF) with a hydrated nutritious gel (HNG) on productive performance, carcass traits, immune variables, and intestinal morphology of broiler chickens. A total of 490 one-day-old Ross 308 broilers were allotted to 5 EF regimens with 7 replicates each. Treatments were as follows: the group deprived of both feed and water during the first 24 hours post-hatch (control); group fed with 2g HNG/bird in chick box and then with 2g HNG + 2g starter feed/bird immediately after placement (T1); group fed with 2g HNG + 2g starter feed/bird in chick box (T2); and the group fed with 2g HNG + 2g starter feed/bird immediately after placement (T4). The results showed that HNG-treated groups had lower relative yolk sac weight compared to the control group ($P < 0.05$). During the starter period, T2, T3, and T4 groups had significantly ($P < 0.05$) higher body weight gain (BWG) and feed intake (FI) than the control group. At 24 d of age, the T2 group had the highest BW, differing significantly from those of the control and T1 groups. During the grower period, the T4 group had higher FI than the control group ($P < 0.05$). Antibody response against infectious bronchitis virus (IBV) was improved in the T1, T2, and T3 groups ($P < 0.05$). The highest relative leg weight was found in the T2 group. In conclusion, the HNG administration in chick box and/or immediately after placement had beneficial effects on the health and performance of broiler chickens.

\textbf{HIGHLIGHTS}

- Receiving the nutritious gel resulted in significant increments in yolk sac absorption.
- Early nutrition with the nutritious gel showed positive effects on weight gain and feed intake during the first 10 d post-hatch.
- On the 25\textsuperscript{th} day after hatch, gel-receiving groups had higher antibody titers against infectious bronchitis virus as compared to the control group.

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\textbf{Introduction}

The first few days after hatch are known as the most crucial phase of a broiler chicken’s life. During these periods of time, dramatic structural, metabolic, and functional evolutions occur in different organs of the body, including digestive, immune, and muscular systems. The pattern of these evolutions is strongly influenced by several intrinsic (e.g. gender, genetic and maternal traits) and extrinsic (e.g. pre- and post-place-ambient conditions, and nutrition) factors, ultimately affecting the ability of the bird to survive and express its maximum genetic potential (Ipek and Sozcu 2015; De Jong et al. 2017; Yerpes et al. 2020, 2021).

Conventionally, the time gap between the hatching and placement in the production house may take up to 24–72 hours, during which the chicks experience a series of stressful events, including early feed and water deprivation, dehydration, handling, vaccination, and transportation (Batal and Parsons 2002; Panda et al. 2015). Being exposed to such stressors, commercially hatched chicks are more likely to have...
insufficiently developed digestive, muscular, and immune systems, leading to poorer performance, health, and welfare (Khosravinia 2015; De Jong et al. 2017; Hedlund et al. 2019).

The time elapsed until the newly hatched chicks have access to the first water and feed has been shown to be negatively correlated with growth performance, intestinal morphology, nutrient uptake rate, and immune response in broilers and laying hens due to dehydration and energy depletion (Shira et al. 2005; Panda et al. 2015; Shinde et al. 2015; Abou-Elnaga and Selim 2018). Metabolic changes caused by delayed access to feed may decrease the development of lymphoid organs, increase the susceptibility of birds to disease, and impair the immune response (Dibner et al. 1998; Shira et al. 2005; Panda et al. 2015), ultimately raising the production costs.

The digestive and immune systems of newly hatched chicks are immature and have not yet reached their full developmental and functional potential (Ravindran and Abdollahi 2021). Early feeding (EF) with readily digestible nutrients is believed to promote the chick’s gastrointestinal tract (GIT) and immune system development (Thaxton & Parkhurst 1976; Batal and Parsons 2002; Ravindran and Abdollahi 2021). In addition, EF may accelerate the acclimation of the GIT and metabolic pathways of the body to the exogenous nutrients instead of the yolk sac nutrients, resulting in better feed efficiency and improved profitability.

The yolk sac (YS) that surrounds the yolk is the first extraembryonic membrane advancing from the embryonic midgut. The membrane plays a crucial role in taking up nutrients and maternal immunoglobulin Y (IgY) that are deposited into the yolk by the breeder hen (Wong and Uni 2021). It has been reported that early chick survivability and quality are directly related to the rate of yolk sac absorption (Wong and Uni 2021).

NEWLY HATCHED CHICKS SUBJECTED TO AN EARLY FEED DEPRIVATION (48–72 h) ARE MORE LIKELY TO UPTAKE THE YOLK SAC MATERIALS SLOWER THAN THEIR IMMEDIATELY FED COUNTERPARTS (NOY ET AL. 1996; EL-HUSSEINY ET AL. 2008). THE EFFECT COULD BE MORE COMPLICATED UNDER EXTREME TRANSPORT DISTANCES AND CONDITIONS (YERPES ET AL. 2021). THE STIMULATING EFFECT OF EF ON YOLK UTILIZATION IS ATTRIBUTED TO THE INCREASED INTESTINAL MOVEMENTS IN THE PRESENCE OF FEED, CAUSING THE YOLK CONTENT TO MOVE THROUGH THE YOLK STALK INTO THE INTESTINE. THE RATE OF YOLK UPTAKE IS POSITIVELY INFLUENCED BY GIT ACTIVITY (NOY ET AL. 1996; SANTOS AND SILVERSIDES 1996).

Exposure of hatchlings to the opportunistic pathogenic microorganisms existing in their surrounding environment is another risk factor that may affect day-old chick quality and subsequent performance. Recent findings have shown that despite the common belief indicating the absence of any microorganism in the GIT of newly hatched chicks, they have a small narrow-spectrum microbial community in their gut primarily transmitted from the breeder flock (Ballou et al. 2016; Kumar et al. 2018). However, the community is intensely delicate and sensitive to the environmental microbiome; the young host, therefore, is highly vulnerable to infectious diseases. Oral administration of beneficial microorganisms or probiotics during the first few days post-hatch may bring advantages for the host by competitive exclusion of pathogens (Nurmi et al. 1992).

All the problems could largely be resolved by oral delivering appropriate compounds such as essential nutrients, moisture, antioxidants, metabolic modifiers, readily available energy substrates, probiotics, and prebiotics immediately after hatch. For this reason, several EF systems have recently been developed, including in ovo feeding (Uni and Ferket 2004), on-farm hatching, and hatchery-fed (Da Silva et al. 2021) systems. However, setting up such systems requires significant upfront investments. The provision of a hydrated nutritious gel (HNG) in the chick box appears to be a cost-effective and easily feasible method. These products can be used to offer a wide variety of readily digestible nutrients and highly effective additives in hatchery tries or day-old chick boxes with a considerable amount of moisture without allowing the chick box and chicks to get wet.

Royal Chick is an innovative nutrient-enriched powder product that forms a jelly mass when mixed with water. As claimed by the manufacturer, the product prevents initial weight loss, alleviates oxidative stress, improves skeletal muscle growth, and stimulates the development of the digestive and immune systems of the newly hatched chicks. This study aimed to evaluate the effect of early feeding with Royal Chick on productive performance, carcass traits, immune system function, and jejunal morphometry of broiler chickens.

Materials and methods

Bird husbandry and experimental design

This study was conducted using 490 one-day-old Ross 308 broiler chickens (initial weight: 43 ± 2 g) in a completely randomized design with 5 treatment groups and 7 replicate pens of 14 birds each (1:1 sex ratio). Each experimental unit was accommodated in a 1.2 m × 1.2 m floor pen equipped with two nipple...
drinkers. A try feeder was used per pen for the first five days after placement and replaced with a 40 cm diameter hanging plastic feeder afterwards. Feed and water were available ad libitum during the rearing period with a lighting program providing 23 hours of light per day. During the first week, the house microclimatic temperature and relative humidity were set at 32–34°C and 60–75%, respectively. Then, the temperature was decreased linearly by 1°C per two days until the final temperature reached 20–22°C.

**Early feeding regimens used in this study**

The nutritious gel used in this study, Royal Chick, contained a synbiotic (prebiotics: *Enterococcus faecium, Pediococcus acidilactici, Bacillus subtilis, Lactiplantibacillus plantarum, Lactobacillus rhamnosus, Lactobacillus casei, and Bifidobacterium bifidum*, and *Saccharomyces cerevisiae*; prebiotic: fructooligosaccharides), an energy source (sucrose), proteins, essential amino acids, vitamins, trace elements (Iron, Copper, Manganese, Selenium, and Zinc), electrolytes (Potassium, Sodium, and Chloride), antioxidants (BHA and BHT), exogenous enzymes and gelatinizing agents (Table 1). According to the manufacturer’s instructions, a dose of 0.0645 g of the dry powder product is required per chick for each time of administration. After calculating and weighing the required amount of the powder, it was mixed into 30 times its own weight of drinking water to form the ready-to-use HNG.

The evaluated EF regimens were: the group deprived of both feed and water during the first 24 hours post-hatch (control group); the group fed with 2 g HNG/bird in chick box and then with 2 g HNG + 2 g starter feed/bird immediately after placement (T1); the group fed with 2 g HNG + 2 g starter feed/bird in chick box (T2); the group fed with 2 g HNG-bird in chick box (T3); and the group fed with 2 g HNG+ 2 g starter feed/bird immediately after placement (T4). All the chicks were kept in chick boxes up to 24 hours post-hatch. Then all experimental units were randomly allocated into the floor pens and reared for 35 days on standard starter (2–11 days), grower (12–24 days), and finisher (25–35 days) diets (Aviagen Inc 2019; Table 2).

**Evaluated parameters**

**Yolk sac absorption and productive performance**

At 2 days of age, 2 representative birds per pen were selected, weighed, and decapitated to determine the yolk sac weight. The yolk sac was weighed with a digital scale (±0.01 g) and results were expressed as a percentage of live body weight. The average body weight (ABW), feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were

| Table 1. Nutrient analysis of Royal Chick gel powder (as fed basis). |
|-----------------|-----------------|
| Crude protein % | 5.50            |
| Methionine + Cystine % | 0.45 |
| Lysine %         | 0.80            |
| Calcium %        | 0.91            |
| Available Phosphorus % | 0.52 |

| Table 2. Ingredients and chemical composition of experimental diets (as fed basis). |
|-------------------------------|-----------------|-----------------|-----------------|
| Ingredients (%)              | Starter (2–11d) | Grower (12–24d) | Finisher (25–35d) |
| Corn                          | 58.30           | 61.41           | 66.43           |
| Soybean meal (CP, 44%)        | 37.40           | 33.88           | 28.61           |
| Soybean oil                   | 0.80            | 1.65            | 2.17            |
| Carbonate calcium             | 1.06            | 0.98            | 0.91            |
| Di calcium phosphate (DCP)    | 1.77            | 1.56            | 1.40            |
| Sodium chloride (NaCl)        | 0.35            | 0.35            | 0.35            |
| Mineral and vitamin premix    | 0.50            | 0.50            | 0.50            |
| DL-methionine                 | 0.34            | 0.29            | 0.27            |
| L-lysine HCl                  | 0.27            | 0.20            | 0.20            |
| L-threonine                   | 0.11            | 0.08            | 0.06            |
| Calculated composition (%)    | 2850            | 2945            | 3040            |
| Metabolizable energy (Kcal/kg)| 21.86           | 20.45           | 18.54           |
| Crude protein (CP)            | 0.91            | 0.83            | 0.75            |
| Calcium                       | 0.46            | 0.41            | 0.38            |
| Available phosphorus          | 0.15            | 0.15            | 0.15            |
| Methionine                    | 0.15            | 0.15            | 0.15            |
| Methionine + Cystine          | 1.03            | 0.94            | 0.86            |
| Lysine                        | 1.37            | 1.23            | 1.10            |
| Threonine                     | 0.92            | 0.84            | 0.74            |

*Supplied per kg of diet: vitamin A as acetate, 8800 IU; Cholecalciferol, 2500 IU; vitamin E (as dl-α tocopherol) 11 IU, vitamin K3, 2.2 mg; Vitamin B12, 0.01 mg; thiamine, 1.5 mg; Riboflavin, 4 mg; Niacin 35 mg, folic acid 0.5 mg; Biotin, 0.15 mg; pyridoxine 2.5 mg; pantothenate, 8 mg; choline chloride, 50 mg; Betaine 190 mg; Zinc, 65 mg; Magnesium, 75 mg; selenium, 0.2 mg; iodide, 0.9 mg; Copper, 6 mg; Iron, 75 mg.
determined at the end of each nutritional phase (11, 24 and 35 d of age).

**Carcass traits**
At 35 days of age, two birds with body weights close to the average weight of the pen were weighed and killed by cervical dislocation. Carcass parts (including legs, breast, abdominal fat, spleen, and bursa of Fabricius) were weighed and, ultimately, the results were expressed as a percent of live body weight. Breast muscle width and length were also measured and reported as a ratio of live body weight.

**Maternal antibody titer and humoral immune response**
At 2 d of age, two birds per replicate were used to take blood samples from the jugular vein. The two samples of each replicate were pooled and centrifuged at 3000 RPM for 15 minutes; then, the obtained serum was analyzed for maternal antibody titers against Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and avian influenza virus (AIV). At 25 d of age, two additional birds per replicate were bled via the brachial vein for the determination of antibody titers against NDV, IBV, and AIV. Antibody titers against NDV and AIV were determined using hemagglutination inhibition assay (Log2), whereas the ELISA technique was used to evaluate humoral immune status against IBV (IDVET kit, France).

**Intestinal morphology**
A 1-cm section from the middle part of jejunum tissue was cut and removed at 35 days of age. Further processing and morphometric assessments of the samples were conducted as described by Khaligh et al. (2019). The measured parameters included villus length, villus width, crypt depth, and lamina propria thickness. The following formula was used to calculate the surface area of the villus (30):

\[
\text{The surface area of the villus} \; (\text{mm}^2) = 2\pi \times \left(\frac{1}{2} \times \text{villus width}\right) \times \text{villus height}
\]

**Statistical analysis**
All raw data were processed using excel software and then subjected to Minitab’s outlier and normality tests. Then, further analysis was performed using SAS (2001) Generalized Linear Model (GLM) procedure. Means were separated using Duncan’s multiple range test (p < 0.05). In addition, the authors compared the mean of a certain treatment group individually with that of the control group using the student’s t-test procedure, since overall ANOVA may sometimes fail to detect the difference between the greatest and smallest means. The statistical model applied to the data was as follows:

\[
y_{ij} = \mu + T_i + e_{ij},
\]

where \(y_{ij}\) represents each of the observations, \(\mu\): the population mean, \(T_i\): the effect of treatments, and \(e_{ij}\): random residual or test error.

**Results**

**Yolk sac absorption and productive performance**
The effects of different EF regimens involving a hydrated nutritious gel on yolk sac relative weight at 2 d of age and productive performance of broiler chickens during different periods are shown in Tables 3–6. The results indicated that regardless of its administration route and time, HNG caused significant reductions in relative yolk sac weight in comparison to the control group (\(p = 0.05\)). Early feeding with a combination of HNG and starter feed in the chick box resulted in a significant improvement in FI during the starter period (\(p < 0.05\)). The groups receiving HNG (except the T4 group) had higher FCR than the control group in the starter period (\(p < 0.05\)). Consumption of HNG in chick box (with or without starter feed; T2 and T3) or immediately after placement (T4) improved the ABW at 11 days of age and increased BWG during the starter period (\(p < 0.05\)). However, HNG administration in the chick box and once again immediately following placement did not affect these parameters in the starter period (Table 3).

The birds that received HNG in a single dose immediately after placement (T4) consumed more feed than the control birds during the grower phase (\(p < 0.05\)). The T2 group had a greater 24-day body weight as compared to the control and T1 groups (\(p < 0.05\)). Nevertheless, FCR was not affected by experimental treatments (Table 4). The employed EF regimens had no effect on growth performance traits during the finisher phase and the whole experimental period (Tables 5 and 6).

**Carcass traits**
The effect of different EF regimens on carcass characteristics of broiler chickens is shown in Table 7.
Table 3. The effect of receiving hydrated nutritious gel (HNG) in chick box (with or without starter feed) and immediately after placement on yolk sac relative weight and productive performance of broiler chickens in the starter period (2–11 d)a.

| Variables                        | Control | T1 | T2 | T3 | T4     | SEMc | P-value |
|----------------------------------|---------|----|----|----|--------|------|---------|
| Relative yolk sac weight (%)     | 3.80b   | 2.67b| 2.46b|1.21b|2.81b   | (11)2.27b | 0.251   | 0.003   |
| Feed intake (g/b/d)              | 222.33c | 224.37b | 235.53a | (6)234.76a | 233.58a | (6)223.78b | 2.451   | 0.001   |
| Body weight gain (g/b/d)         | 21.56c  | 22.05c | 23.16a | (6)22.61c | 22.78ab | (6)21.88c | 0.274   | 0.002   |
| Feed conversion ratio            | 18.59b  | 18.63b | 19.65a | (6)19.36a | 19.47b | (6)14.17bc | 0.237   | 0.006   |

a,b&c Means within a row with no common lowercase superscripts differ significantly (P < 0.05).

The means related to relative yolk sac weight represent the 14 observations, but, the body weight, feed intake, weight gain and feed conversion ratio represent 7 observations, except for the ones above which the associated number of observations is written in parentheses on their left side.

*T1: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement; T2: 2 g HNG + 2 g starter feed/bird in chick box; T3: 2 g HNG/bird in chick box; T4: 2 g HNG + 2 g starter feed/bird immediately after placement.

SEM: standard error of the mean.

Table 4. The effect of receiving hydrated nutritious gel (HNG) in chick box (with or without starter feed) and immediately after placement on productive performance of broiler chickens in the grower period (12–24 d)a.

| Variables                        | Control | T1 | T2 | T3 | T4 | SEMc | P-value |
|----------------------------------|---------|----|----|----|----|------|---------|
| Live body weight (g/b)           | 958.40c | 952.50b | (6)990.60a | 975.56b | (6)978.40ab | 9.335 | 0.050   |
| Feed intake (g/b/d)              | 73.76c  | 72.99c | 74.84ab | 73.93bc | (6)75.81a | 0.597 | 0.029   |
| Body weight gain (g/b/d)         | 52.52bc | 52.01c | 53.27ab | 53.30bc | (6)53.19a | 0.582 | 0.433   |
| Feed conversion ratio            | 1.40    | 1.40 | 1.41 | (6)1.40 | 1.41 | (6)1.41 | 0.012 | 0.702   |

a,b&c Means within a row with no common lowercase superscripts differ significantly (P < 0.05).

The means related to body weight, feed intake, weight gain and feed conversion ratio represent 7 observations, except for the ones above which the associated number of observations is written in parentheses on their left side.

*T1: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement; T2: 2 g HNG + 2 g starter feed/bird in chick box; T3: 2 g HNG/bird in chick box; T4: 2 g HNG + 2 g starter feed/bird immediately after placement.

SEM: standard error of the mean.

Table 5. The effect of receiving hydrated nutritious gel (HNG) in chick box (with or without starter feed) and immediately after placement on productive performance of broiler chickens in the finisher period (25–35 d)a.

| Variables                        | Control | T1 | T2 | T3 | T4 | SEMc | P-value |
|----------------------------------|---------|----|----|----|----|------|---------|
| Live body weight (g/b)           | 1931.12 | 1957.26 | 1992.17 | 1994.26 | (6)1990.54 | 21.762 | 0.182   |
| Feed intake (g/b/d)              | 150.51bc | 150.44c | 152.41 | 153.79 | (6)153.25 | 1.777 | 0.566   |
| Body weight gain (g/b/d)         | 89.80bc | 91.34c | 91.78 | 92.61 | (6)92.01 | 1.602 | 0.773   |
| Feed conversion ratio            | 1.68    | (6)1.66 | 1.66 | 1.66 | (6)1.67 | 0.018 | 0.941   |

a,b&c Means within a row with no common lowercase superscripts differ significantly (P < 0.05).

The means related to body weight, feed intake, weight gain and feed conversion ratio represent 7 observations, except for the ones above which the associated number of observations is written in parentheses on their left side.

*T1: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement; T2: 2 g HNG + 2 g starter feed/bird in chick box; T3: 2 g HNG/bird in chick box; T4: 2 g HNG + 2 g starter feed/bird immediately after placement.

SEM: standard error of the mean.

Table 6. The effect of receiving hydrated nutritious gel (HNG) in chick box (with or without starter feed) and immediately after placement on productive performance of broiler chickens in the finisher period (25–35 d)a.

| Variables                        | Control | T1 | T2 | T3 | T4 | SEMc | P-value |
|----------------------------------|---------|----|----|----|----|------|---------|
| Feed intake (g/b/d)              | 82.55   | (6)82.10 | 84.90 | 83.69 | (6)84.35 | 0.848 | 0.200   |
| Body weight gain (g/b/d)         | 54.11   | 54.83 | 55.80 | 55.81 | (6)55.76 | 2.595 | 0.420   |
| Feed conversion ratio            | (6)1.53 | (6)1.50 | 1.52 | 1.50 | (6)1.51 | 0.001 | 0.490   |

a,b&c Means within a row with no common lowercase superscripts differ significantly (P < 0.05).

The means related to body weight, feed intake, weight gain and feed conversion ratio represent 7 observations, except for the ones above which the associated number of observations is written in parentheses on their left side.

*T1: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement; T2: 2 g HNG + 2 g starter feed/bird in chick box; T3: 2 g HNG/bird in chick box; T4: 2 g HNG + 2 g starter feed/bird immediately after placement.

SEM: standard error of the mean.

According to the results, the relative weight of the leg was significantly improved in the T1, T2, and T3 groups when compared to the control group (P < 0.05). Other carcass parameters were not affected by the experimental treatments.

**Maternal antibody titers**

The effects of different EF regimens on maternal antibody titers against NDV, IBV, and AIV in broiler chickens are shown in Table 8. We found no significant...
effect of EF on maternal immunity variables; The HNG-treated groups, however, mostly showed numerical increments in the maternal antibody titers compared to the untreated control group.

**Humoral immune response and relative weight of immune organs**

The effects of different EF regimens on humoral immune response (25 d of age) and relative weight of immune organs (35 d of age) in broiler chickens are shown in Table 9. According to the table, the T2 group had a higher IBV-specific antibody titer than the control group ($P < 0.05$). Also, when treatment groups were individually compared with the control group using the $t$-test procedure, T1 ($P = 0.045$) and T3 ($P = 0.017$) groups displayed significant improvements in antibody titer against IBV. The relative weights of the spleen and bursa of Fabricius were not affected by the experimental treatments.

**Intestinal morphology**

The effect of different EF regimens on intestinal morphology in broiler chickens is shown in Table 10. The regimens had no significant effect on jejunal morphological characteristics in 35 days-old broiler chickens ($P > 0.05$).

**Discussion**

**Yolk sac absorption and productive performance**

Increased yolk sac absorption and improved performance in HNG-receiving groups (especially in the starter period) were mainly in line with our expectations and the findings of other researchers. Yang et al. (2009) suggested that long-term feed deprivation decreased the growth performance and the yolk sac absorption in newly hatched goslings. El-Husseiny et al. (2008) reported that feed deprivation decreased growth performance over the first 2 d post-hatch period and the rate of yolk sac absorption was greater in fed than in fasted chicks on post-hatch days 1 and 2.

The stimulating effect of EF on yolk utilization may be attributed to the increased intestinal movements in the presence of feed, causing the yolk content to move through the yolk stalk to the duodenum. The existence of a positive correlation between the rate of yolk uptake and gastrointestinal movements has been described previously (Noy et al. 1996; Santos and Silversides 1996).
The increased yolk sac absorption in HNG-treated chicks may also be related to the probiotic microorganisms used in the gel formula. In a recent study by Khaligh et al. (2019), in ovo delivery of probiotic bacteria (a combination of *Lactobacillus salivarius* and *Lactobacillus plantarum*) led to a significant reduction in yolk sac weight in newly hatched broiler chicks compared to intact un-injected eggs and eggs injected with distilled water (*P* < 0.05). Probiotics stimulate gut motility by producing short-chain fatty acids and releasing GIT regulatory peptides including YY polypeptide (Gibson and Roberfroid 1995; Cherbut et al. 1998; Dass et al. 2007). Some bacteria can decarboxylate tryptophan to tryptamine which induces the secretion of serotonin by enterochromaffin cells and increases gut motility (Takaki et al. 1985). Thus, in the current study, the presence of probiotics in HNG may increase yolk sac absorption by inducing intestinal motility.

A meta-analysis was conducted by De Jong et al. (2017) to evaluate the effects of post-hatch feed and water deprivation on the performance, growth performance, and welfare of broiler chickens. The authors concluded that post-hatch feed and water deprivation has substantial deteriorative effects on the performance and survivability of broiler chickens and these effects are likely to be exacerbated with increasing duration of the deprivation period. They also suggested that ≥48 hours post-hatch feed and water deprivation has long-term adverse effects on the growth and Survival of broiler chickens. In the current study, the positive effect of EF on performance traits was only observed up to 24 d of age and tended to disappear afterward. This may be due to the shorter deprivation period (24 h) to which the control group was exposed (De Jong et al. 2017). The low persistence of the positive effects of the EF regimens in the present study may also be related to the age of the breeder hens from which the experimental chicks were provided. Ipek and Sozcu (2015) reported that the incubation period lasted for 510 h in the young breeder flock (33 weeks old), whereas it was accomplished in 518 h in the old breeder flock (62 weeks old). The extended incubation period causes the early-hatching chicks to suffer greater degrees of dehydration and environmental stressors. Since the chicks

### Table 9. The effect of receiving hydrated nutritious gel (HNG) in chick box (with or without starter feed) and immediately after placement on maternal antibody titer against Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and avian influenza virus (AIV) and relative weight of immune organs in broiler chickens (25 d)

| Variables                  | Control | T1: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement | T2: 2 g HNG + 2 g starter feed/bird in chick box | T3: 2 g HNG/bird in chick box | T4: 2 g HNG + 2 g starter feed/bird immediately after placement | P-value | SEM |
|----------------------------|---------|-----------------------------------------------------------------------------------------------|-----------------------------------------------|-------------------------------|----------------------------------------------------------------|---------|-----|
| NDV (HI; Log2)             | 5.29    | 5.86                                                                                          | 5.93                                                                                     | 6.00                                                                 | *(12)* 5.92 | 0.281 | 0.373 |
| AIV (HI; Log2)             | 4.21    | 4.07                                                                                          | 3.93                                                                                     | 4.29                                                                 | *(12)* 3.33 | 0.118 | 0.107 |
| IBV                        | 1127.60b| 1728.90ab                                                                                     | 2263.10*                                                                                 | 1774.50ab                                                                 | *(12)* 1484.00b | 245.86 | 0.027 |
| Bursa of Fabricius (%)     | 0.19    | 0.21                                                                                          | 0.18                                                                                     | 0.21                                                                 | *(12)* 0.20 | 0.011 | 0.284 |
| *T*-Test                   | Control | T1                                                                                           | T2                                                                                       | T3                                                                     | Control × T3       |         |
| P-value                    | 0.045   | 0.017                                                                                          |                                             |                                                                      |                                                                 |         |

*a* & *b* Means within a row with no common lowercase superscripts differ significantly (*P* < 0.05).

Each mean represents 14 observations, except for the ones above which the associated number of observations is written in parentheses on their left side.

*T1*: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement; T2: 2 g HNG + 2 g starter feed/bird in chick box; T3: 2 g HNG/bird in chick box; T4: 2 g HNG + 2 g starter feed/bird immediately after placement.

**SEM**: standard error of the means.

### Table 10. The effect of receiving hydrated nutritious gel (HNG) in chick box (with or without starter feed) and immediately after placement on the intestinal histomorphology of broiler chickens (35 d)

| Variables                  | Control | T1: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement | T2: 2 g HNG + 2 g starter feed/bird in chick box | T3: 2 g HNG/bird in chick box | T4: 2 g HNG + 2 g starter feed/bird immediately after placement | P-value | SEM |
|----------------------------|---------|-----------------------------------------------------------------------------------------------|-----------------------------------------------|-------------------------------|----------------------------------------------------------------|---------|-----|
| Villus height (μm)         | 1698.00 | 1712.29                                                                                       | 1753.14                                                                                   | 1772.00                                                                 | *(12)* 1478.20 | 85.140 | 0.147 |
| Villus width (μm)          | 186.61  | 210.71                                                                                         | 213.14                                                                                     | 192.71                                                                 | *(12)* 211.17 | 12.794 | 0.450 |
| Crypt depth (μm)           | *(12)* 17.23 | 246.43                                                                                       | 227.14                                                                                     | 245.86                                                                 | *(12)* 238.50 | 13.327 | 0.468 |
| Thickness of lamina propria (μm) | 43.00 | 40.71                                                                                         | 39.71                                                                                     | 45.86                                                                 | *(12)* 39.53 | 1.903  | 0.108 |
| Villi surface area (mm²)   | 0.92    | 1.24                                                                                          | 1.17                                                                                       | 1.08                                                                 | *(12)* 1.02 | 0.090  | 0.342 |
| Villus height/crypt depth  | 7.78    | 7.12                                                                                          | 8.00                                                                                       | 7.64                                                                 | *(12)* 6.17 | 0.511  | 0.126 |

Each mean represents 14 observations, except for the ones above which the associated number of observations is written in parentheses on their left side.

*T1*: 2 g HNG/bird in chick box and 2 g HNG + 2 g starter feed/bird immediately after placement; T2: 2 g HNG + 2 g starter feed/bird in chick box; T3: 2 g HNG/bird in chick box; T4: 2 g HNG + 2 g starter feed/bird immediately after placement.

**SEM**: standard error of the means.
used in this experiment were from a young breeder flock (34 weeks), they may not experience severe dehydration and stress. As a result, early hydration of the chicks with HNG induced only short-term positive effects on performance traits.

**Carcass traits**

In the present investigation, EF with HNG (except T4 regimen) was accompanied by improvements in leg muscle yield. Abousekken et al. (2017) obtained higher carcass yield, deboning ratio, thigh meat ratio, and breast meat ratio in early-fed broilers than the control birds. Post-hatch mitotic activity of satellite cells (SCs) is one of the most important events in skeletal muscle development. Researchers have shown that EF immediately after hatching stimulates the SC mitotic activity in the muscle and improves muscle weight (Moore et al. 2005). Post-hatch development of skeletal muscle is largely dependent on the proliferation of mononuclear SCs located between the basal lamina and microfiber sarcolemma (Kuang et al. 2007). Satellite cells play an essential role in muscle regeneration and increase cell nuclei in the tissue (Kawano et al. 2008). They also contribute to muscle fibre hypertrophy during the post-hatch life (Yablonka-Reuveni 2011). Satellite cells can fuse with existing muscle fibres or form new myofibers (Starkey et al. 2011).

**Maternal antibody titers**

Any increase in early yolk sac uptake is expected to be associated with improved maternal immunity since the yolk sac is the primary source of the circulating antibodies in young chicks; however, in the present study, despite the increased yolk sac uptake in the EF groups, only numerical elevations were observed in maternal antibody titers. Moran and Reinhart (1980) reported that fasted chicks showed a slower rate of yolk uptake and favored absorbing water and lipid fractions compared to protein while the converse was true in EF chicks. Owing to the proteinaceous nature of antibodies, EF is anticipated to promote the transfer of maternal immunoglobulins from the yolk sac to the systemic circulation. De Castro Vargas et al. (2009) reported that a 12-hour delay in feed access resulted in a significant reduction in antibody titer against NDV at 7 days of age, while maternal antibody titers against IBV and infectious bursal disease (IBD) were not affected. Contradictions in the findings of various studies may be attributed to different factors including the nature of the materials used for early feeding and the age of the bird at the time of blood sampling. De Castro Vargas et al. (2009) used a complete starter feed for EF and collected blood samples at 7 d of age, whereas in the present study, EF-chicks were provided with a hydrated nutritious gel containing more than 96% moisture and bleeding was done at 2d of age.

**Humoral immune response and relative weight of immune organs**

As described previously, concurrent access to HNG and solid complete feed in the chick box resulted in a significantly improved acquired immunity against IBV. Long-term feed and water deprivation during the first few days post-hatch causes dehydration and body energy depletion in chicks. Delayed access to feed causes reduced growth, increased mortality, poor response to vaccination, reduced development of the gastrointestinal tract and immune system, and diminished disease resistance (Panda et al. 2015). Early feeding is one of the effective solutions to promote the development of the immune system in newly hatched chicks (Juul-Madsen et al. 2004).

Selim et al. (2021) found no positive effect of early post-hatch feeding on the growth of lymphoid and digestive organs of 14d-old chickens. Histomorphological examinations showed that the EF groups had the higher thickness of cortex and cortex to medulla ratio of thymus and bursa compared to the fasted ones. Germinal center areas and white pulp of the spleen increased in the EF chicks, suggesting the enhanced proliferation and maturation of B-cells in the secondary lymphoid organs. The authors also discovered that the majority of the EF chicks displayed up-regulated expression of splenic-immune-related genes. Abbasi et al. (2016) found that in ovo injection of vitamin K3 increases antibody titer against sheep red blood cells (SRBC) at 35 and 42 days of age. Thus, EF may induce long-term positive effects on immune system function.

**Intestinal morphology**

The findings of the current study regarding intestinal morphology were inconsistent with the results of Abou-Elnaga and Selim (2018). They found that EF with a protein-rich or molasses-rich diet improved intestinal function and development in laying hens. Biloni et al. (2013) reported that 24-hour water and feed deprivation (compared to feeding 4 hours after hatching) significantly decreased villus height of the duodenum and jejunum. Discrepancies observed
between our results and the findings of other researchers may be derived from the time of tissue sampling; we collected intestinal tissue samples from 35-d-old broilers, whereas Abou-Elnaga and Selim (2018) and Biloni et al. (2013) had collected the samples from younger birds (<14d of age). Adeleye et al. (2018) indicated that the duration of the deprivation period, the assessed area of the intestine, and the strain of birds under study are additional variance sources of data published on the effect of EF on gut morphology.

Conclusions
In conclusion, early feeding of broiler chickens using the hydrated nutritious gel (Royal Chick) with or without a solid starter feed had an accelerative effect on yolk sac uptake. Growth performance, thigh muscle yield, and humoral immune responses were also positively affected by the EF regimens. Maternal antibody titers and gut morphological variables were not influenced by the experimental treatments. Further investigations are necessary to identify possible interactions of different EF regimens involving HNG with breeder age, deprivation duration, and pre-placement environmental conditions on subsequent performance and health status in broiler chickens.

Notes
1. Manufacturer: Tehran Toyur Sabz Andishan Bartar Company, Mashhad, Razavi Khorasan, Iran
2. Brand name: Bio-Poul; Manufacturer: Zist Darman Mahan Company, Tehran, Iran
3. Brand name: GREEN; Manufacturer: Shandong Green New Materials Co., Shandong, China
4. Brand name: Hilmar® 8010 Whey protein isolate; Manufacturer: Hilmar Cheese Company, Hilmar, California, U.S.A
5. Brand name: AminTotal; Manufactured for Laprovet by Ceva Sante’ Animale 3 avenue des crypre’s- 53950 Louverne- France
6. Brand name: Maxidan®; Manufacturer: Miavit GmbH, Esstxe, Germany
7. Brand name: Rovabio® Excel; Adisseo Company; PiGdk – Snekkebaek, Klintevej 133, DK-4780 Stege, France
8. Manufacturer: Tehran Toyur Sabz Andishan Bartar Company, Mashhad, Razavi Khorasan, Iran

Ethical approval
All the experimental procedures were reviewed and approved by the Animal Ethics Monitoring Committee of Agricultural and Natural Resources Research and Education Centre, Mashhad, Razavi Khorasan Province, Iran.

Disclosure statement
No potential conflict of interest was reported by the author(s).

ORCID
Farhad Khaligh http://orcid.org/0000-0003-4302-3416

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