Effects of methionine and/or disaccharide injected in the amnion of geese on post-hatching pectoral muscle and small intestine development, glycogen reserves, jejunum morphology, and digestive enzymes activities

De Xin Dang,[*,†] Haizhu Zhou,[‡] Yujie Lou,[‡] and Desheng Li[*,1]

[*]*College of Animal Science and Veterinary Medicine, Jinzhou Medical University, Jinzhou 121001, China;* [†]*Department of Animal Resources Science, Dankook University, Cheonan 31116, South Korea; and* [‡]*College of Animal Science and Technology, Jilin Agricultural University, Changchun, Jilin, China*

ABSTRACT This study was conducted to investigate the effects of in ovo injection of methionine (**Met**) and/or disaccharide (**DS**) on post-hatching pectoral muscle and small intestine development, glycogen reserves, jejunum morphology, and jejunum digestive enzymes activities. A total of 600 fertilized eggs containing live embryo from geese were randomly assigned into 4 groups with 6 replicates and 25 eggs per replicate in a completely randomized design employing a 2 × 2 factorial experiment. Factors in 4 groups included noninjection, Met injection (5 g/L Met + 7.5 g/L NaCl), DS injection (25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl), or DS plus Met injection (25 g/L maltose + 25 g/L sucrose + 5 g/L Met + 7.5g/L NaCl), respectively. In ovo nutritional injections were performed at day 23 of incubation, and the experiment until d 21 post-hatching. We found that in ovo feeding of Met increased relative weight of pectoral muscle and small intestine, jejunum alkaline phosphatase activities, and jejunum villus height and surface area. DS injection improved the relative weight of pectoral muscle, pectoral and liver glycogen contents, jejunum villus height, width, and surface area, and jejunum sucrase, Na^+/K^+ATPase, and alkaline phosphatase activities. In addition, Met plus DS injection synergistically improved jejunum villus height and surface area. Therefore, Met plus DS injection is a suitable strategy for improving intestinal parameters in gosling during post-hatching periods.

Key words: goose, in ovo injection, intestinal health, post-hatching development, nutrient absorption

2022 Poultry Science 101:101867
https://doi.org/10.1016/j.psj.2022.101867

INTRODUCTION

Commercial poultry hatcheries are common delays to remove birds from the hatching incubator in order to make the maximum number of eggs hatched (Willemsen et al., 2010), which inevitably prolongs the time for receiving feed and water access at the initial stage of life (Zamani et al., 2018). Additionally, hatchery treatments and/or transportation in modern commercial poultry farming are also subject to delays the access to feed and water for birds (Nouri et al., 2018). Because the initial stage after incubation in birds is a period with a relatively high metabolic rate, the requirements of energy and protein are increased (Elwan et al., 2019). The existence of the fasting period leads to a deficiency of energy and protein supply, resulting in the limited growth of birds in the initial crucial period of life (Kanagaraju and Rathnapraba, 2019).

The geese are seasonal producers, eggs of geese are mainly used for reproduction and research purposes (Tserveni-Goussi and Fortomaris, 2011). Therefore, the improvement of gosling quality during post-hatching stages in commercial geese farming becomes critical (Baykalir et al., 2021).

In ovo injection technique provides a suitable way for helping birds pass the fasting period (Kadam et al., 2013). The technique of delivering various nutrients, supplements, immunostimulants, vaccines, and drugs via the in ovo route is gaining wide attention among researchers for boosting the production performance and immunity and for safeguarding the health of poultry (Saeed et al., 2019; Alagawany et al., 2021; Hassan et al., 2021). Amnion has proven to be an effective site for implementing embryo injection (Peebles, 2018). The growth and development of embryo could be uplifted by injecting nutrients into the amnion (Jha et al., 2019). Digestible disaccharides (**DS**) as a suitable candidate for providing exogenous energy plays

Received February 18, 2022.
Accepted March 13, 2022.

[1]Corresponding author: lidesheng0726521@126.com

an important glucose precursor for late-term bird embryo catabolism, could alleviate the energy deficiency through stimulating glucose metabolism (Chen et al., 2010b). Methionine (**Met**) as the first limiting amino acid for poultry has many physiological functions, like giving the methyl group a vital methyl donor (Reda et al., 2020; Elwan et al., 2021), ameliorating oxidative stress, and uplift the protein supply in embryo (Elnesr et al., 2019). In our previous study, feeding geese embryo with DS and/or Met solution had positive effects on the development of embryo (Dang et al., 2022).

However, studies on the effects of DS and/or Met injected in the amnion of geese embryo on the performance of gosling at post-hatching are still limited. We hypothesized that the delivery of DS and/or Met into the amnion of geese embryo may serve as a tool to provide energy for small intestine and embryo activity, in turn alleviating energy lack, improving pectoral muscle and intestine development. In order to test this hypothesis, we conducted this study to investigate the effects of in ovo injection of DS and/or Met on pectoral muscle development, small intestine parameters, glycogen reserves, jejunum morphology, and digestive enzymes activities.

MATERIALS AND METHODS

Experimental Design, Animals, and Housing

A total of 1,000 eggs from Jilin White geese were obtained from a commercial certified hatchery (Dekun Poultry Food Co., Ltd, Meihekou, Jilin). All eggs were laid on the same day, represented in the same weight class. Fertilized geese eggs were incubated in the standard condition in an incubator (Keyu CFZ microcomputer automatic incubator, Dezhou, Shandong). Before transiting to the incubator, eggs were pre-heated to 30°C for 12 h, disinfected with 37% formaldehyde and potassium permanganate (2:1), and distributed into incubator tray levels. The incubation period included 3 phases (phase 1, d 1−14; phase 2, d 15−28, phase 3, d 29−31). During phase 1, the temperature was 38°C and the humidity was 65%; during phase 2, the temperature was 37.5°C and the humidity was 55%; during phase 3, the temperature was 37.2°C and the humidity was 70%. All eggs were turned once per two hours for 180 s.

On day 23 of incubation, all eggs were candled to select embryonated eggs and the unfertilized or nonviable eggs were removed. After that, a total of 600 fertilized geese eggs containing live embryo were randomly assigned to each of 4 pre-specified treatment groups on each of 6 replicate tray levels (25 eggs per treatment in each tray level). This study used a completely randomized design comprising a 2 × 2 factorial arrangement of treatments. Factors involved were in ovo injection of DS or Met. The groups were divided as: noninjection (control); DS injection; Met injection; or DS + Met injection. The DS solution included 25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl. The Met solution included 5 g/L Met + 7.5 g/L NaCl. The DS plus Met complex solution included 25 g/L maltose + 25 g/L sucrose + 5 g/L Met + 7.5 g/L NaCl. All injected solutions were freshly prepared on the day of injection. All injection solution and paraffin were sterilized under 121°C for 15 min and then allowed to reach room temperature (30°C) before injection. In ovo delivery of substances into the eggs was carried out on d 24 of incubation.

Each egg was cleaned using 70% ethanol and positioned in a holder with the large end on top. The amnion in the in ovo injection group was identified by candling. The upper side of the eggs (the air space) was pierced by an egg-shell punch. Injections were performed with a disinfected injector. A 1.5 mL of each solution was injected into the amnion of each egg to a depth of 20 mm, without hurting the embryo. After each inoculation, the needle was routinely disinfected to minimize the risk of infection. All eggs were held outside the incubator for less than 5 min while injecting, including the noninjected control eggs. Immediately after the injection, the hole was sealed using paraffin, the eggs were returned to the incubator and incubated in line with the routine procedure until hatch.

One-day-old birds were transported to the farm and distributed into cages according to the replicates. Birds were fed the diet (Table 1) beginning as soon as they were placed in the cages. The geese were raised until 28 d of age. During this period, all birds were kept under uniform management conditions according to their treatments. All geese were housed in a temperature-controlled room with continuous lighting. The temperature of the room was maintained at 24°C and then reduced

Table 1. Composition and nutrient levels of the experimental basal diet, (%, as-fed basis).

Ingredients, %	
Corn	60.00
Soybean meal	29.11
Wheat bran	6.00
Fish meal	2.00
Lysine-HCl	0.20
Methionine	0.23
Dicalcium phosphate	0.84
Limestone	0.82
Sodium chloride	0.30
Vitamin and trace mineral premix[1]	0.50
Total	100.00
Calculated value, %	
Metabolizable energy, MJ/kg	11.67
Available phosphorus	0.40
Analyzed composition, %	
Crude protein	19.78
Methionine	0.50
Total sulfate amino acid	0.77
Lysine	1.08
Calcium	0.78
Crude fiber	0.31
Neutral detergent fiber	1.09
Acid detergent fiber	0.35

[1]Provided per kg of complete diet: vitamin D_3, 200 IU; vitamin A (retinyl acetate), 1,500 mg; vitamin E (DL-α-tocopheryl acetate), 12.5 mg; vitamin K_3, 1.5 mg; thiamine, 2.2 mg; riboflavin, 5 mg; nicotinic acid, 65 mg; folic acid, 1 mg; pantothenic acid, 15 mg; pyridoxine, 2 mg; biotin, 0.2 mg; choline, 1,000 mg; Fe, 90 mg; Cu, 6 mg; Mn, 85 mg; Zn, 85 mg; I, 0.42 mg; Se, 0.3 mg; Co, 2.5 mg.

by 2°C per week to a final temperature of 20°C. The sex of geese was not determined in this experiment.

This study was conducted under the supervision of the Animal Care and Use Committee of Jilin Agricultural University (Changchun, China).

Feed Analysis

After homogeneous mixing, feed samples were collected from each dietary group. All feed samples were dried in a 70°C constant temperature oven for 72 h. Subsequently, feed samples were ground and sieved with a 1-mm sieve. Collect feed powder with a diameter of less than 1-mm for feed composition analysis. According to the procedure established by the Association of official analytical chemists (2000), the dry matter (method 930.15), crude protein (nitrogen × 6.25; method 968.06), calcium (method 984.01), and crude fiber (method 991.43) composition in the diet were analyzed. Then, the representative feed samples in each group were hydrolyzed with 6 N HCl for 24 h at 110°C. An amino acid analyzer (2690 Alliance, Waters, Inc., Milford, MA) was used for determining amino acid contents in the diet. In addition, the contents of neutral detergent fiber and acid detergent fiber in the diet were measured according to the method provided by Mertens (2002).

Sample Collection

On d 1, 14, and 21 post-hatching, 3 birds were randomly selected from each replicate group, weighed, and slaughtered by cervical dislocation for measuring relevant parameters.

The liver was removed from the carcass and the adherent material of the liver was carefully removed under ice-cold saline. The sample of the liver was frozen as aliquots in liquid nitrogen, and stored at −80°C for measuring the liver glycogen contents.

One side of the pectoral muscle was weighed and stripped frozen as aliquots in liquid nitrogen after being slaughtered and stored at −80°C for measuring pectoral muscle glycogen contents.

The whole small intestine was removed and the adherent material of the small intestine was carefully removed under ice-cold saline, weighed, and separated into the duodenum, jejunum, and ileum. About the 1-cm long segment from the middle of jejunum were taken in duplicate and placed in 2 separate tubes. One sample was fixed with 10% neutral-buffered formalin solution for histology and the other sample was frozen in liquid nitrogen, and then stored at −80°C for measuring digestive enzymes activities.

Experimental Parameters Measurement

Pectoral Muscle Parameters. The relative weight of breast muscle analysis.

The relative weight of pectoral muscle was calculated using the following equation:

$$Organ\ index\ =\ \frac{Organ\ weight}{Live\ body\ weight}\ \times\ 100\ \%.$$

Glycogen Reserves Analysis. About 0.1 grams sample of liver and pectoral muscle were stored at 1 mL of 8% $HClO_4$, homogenized (in ice) for 45 s, and centrifuged at 7,700 rpm at 4°C for 16 min. A 10-μL aliquot of the supernatant was transferred to a clean polypropylene tube, along with 0.4 mL of 8% perchloric acid and 2.6 mL of iodine color reagent made of 1.3 mL of solution A (0.26 g iodine + 2.6 g potassium iodide dissolved in 10 mL of distilled water) in 100 mL of 67.8% saturated calcium chloride. All samples were read at a wavelength of 450 nm. The amount of glycogen present in sample solution was determined by preparation of a known glycogen standard curve.

Small Intestine Parameter Analysis. The relative weight of small intestine was calculated using the following equation:

$$Organ\ index\ =\ \frac{Organ\ weight}{Live\ body\ weight}\ \times\ 100\ \%.$$

Jejunum Morphology Analysis. The jejunum samples were cut into small pieces and fixed with 10% neutral buffered formalin for 12 h, followed by dehydration in increasing concentrations of alcohol (70, 80, 90, 95, and 100%) and xylene. Consequently, samples were embedded in paraffin and stored in an oven at 60°C. Twelve hours later, samples were removed from the oven and histological cassettes. Fragments were placed in "paper boxes" and covered with paraffin. After the paraffin solidified into blocks, the "papers" were removed and the blocks were kept under refrigeration until the cuts were realized (Felício et al., 2013).

Serial tissue sections (5 μm thickness) were excised perpendicular to the direction of the myofibers using a cryostat. After sectioning, put the paraffin section ribbon on the coating slide glass. Dried slides were kept in oven with 60°C for 2 h to eliminate any excess paraffin. The next step consisted of paraffin removal and slide hydration, using xylene, different concentrations of ethanol. Samples were then stained following the hematoxylin and eosin staining protocol (Felício et al., 2013).

Samples were then dehydrated again and mounted. In each specimen, the villus height and width were measured under a light microscope equipped with a ScopePhpto (LY-WN 300, Hangzhou Scopetek Opto-Eletric Co., Ltd., China). A minimum of 5 measurements per slide were made for each parameter and averaged into one value (Tako et al., 2004). Villus surface area was calculated from the villus height (from the tip of the villi to the villus crypt junction) and width at half height (Uni et al., 1998). Values presented are means from 10 adjacent villi and only vertically oriented villi were measured.

Digestive Enzymes Activities Analysis. Enzyme activities were assayed in homogenized jejunal tissue.

Samples were thawed at 4°C and homogenized in 10 times of the volume of cold normal saline. The homogenates were then centrifuged at 20,000 × *g* for 20 min at 4°C and the supernatant was collected for enzyme assays. Sucrase (Enzyme Commission [EC] 3.2.1.48) was assayed colorimetrically using sucrose as substrates (Dahlqvist, 1984). The activity was expressed as micromoles of glucose released per minute per gram of jejunal wet tissue. Alkaline phosphatase (EC 3.1.3.1) activity was determined by measuring the hydrolysis of p-nitrophenol at 37°C according to Palo et al. (1995) and the unit of activity was expressed as per minute per gram of jejunal wet tissue. Na^+/K^+ATPase (EC 3.6.1.3) activity was determined by measuring the liberation of phosphate from ATP-Na2 (No. A7699, Sigma-Aldrich, St. Louis, MO) in 2 media: medium I (all ATPases system); medium II (Na^+/K^+ATPase restrained system) according to the description by Wheatly and Henry (1987) and the activity of Na^+/K^+ATPase was calculated as the difference between phosphates liberated by each homogenate in the 2 media and was expressed as micromoles of phosphates per milligram homogenate protein or per milliliter of serum per hour.

Statistical Analysis

The data were analyzed as a two-way ANOVA factorial arrangement of treatments using the GLM procedure in SPSS18.0 software, with treatment as the fixed effect and the replicate cage as the experimental unit. Factors involved were in ovo injection of DS and/or Met. The data are presented as the means ± standard deviation. Results were considered significant at $P < 0.05$.

RESULTS

The relative weight of pectoral muscle was increased by in ovo injection of Met ($P < 0.01$) or DS ($P < 0.01$) at d 1 post-hatching (Table 2).

In ovo injection of DS increased pectoral ($P < 0.01$) and liver ($P < 0.01$) glycogen contents at d 1 post-hatching, whereas Met delivery had no significant effects on the glycogen reserves (Table 3).

Met injection increased the relative weight of the small intestine ($P < 0.01$). However, DS injection had no significant effects on the relative weight of the small intestine (Table 4).

In ovo feeding of Met increased the jejunum villus height and surface area at d 1 ($P < 0.01$; $P < 0.01$), 14 ($P < 0.01$; $P < 0.01$), and 21 ($P < 0.01$; $P < 0.01$) post-hatching. DS injection had positive effects on the villus height ($P < 0.01$), width ($P < 0.05$), and surface area ($P < 0.01$) at d 1 post-hatching. Additionally, a synergistic effect of in ovo injection of Met plus DS on the jejunum villus height ($P < 0.05$) and surface area ($P < 0.05$) was observed at d 1 post-hatching in this study (Table 5).

The effects of in ovo injection of Met and/or DS on the activities of the digestive enzymes were shown in Table 6. Met injection increased the activity of jejunum alkaline phosphatase at d 1 post-hatching ($P < 0.01$). DS

Table 2. Effects of in ovo injection of methionine (Met) and disaccharide (DS) on pectoral muscle parameters of geese during the early-life period of post-hatching[1,2].

					P-value		
	Control	DS	Met	DS + Met	Met	DS	Met × DS
Relative weight of pectoral muscle, %							
D 1 post-hatching	0.74 ± 0.07	0.94 ± 0.07	0.88 ± 0.09	1.09 ± 0.06	<0.001	<0.001	0.913
D 14 post-hatching	0.94 ± 0.16	0.92 ± 0.10	0.92 ± 0.05	0.94 ± 0.04	0.973	0.960	0.583
D 21 post-hatching	0.83 ± 0.06	0.84 ± 0.07	0.86 ± 0.04	0.88 ± 0.04	0.137	0.440	0.719

[1]The data are presented as the means ± standard deviation.
[2]Factors in four groups included noninjection (control), DS injection (25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl), Met injection (5 g/L Met + 7.5 g/L NaCl), or DS + Met injection (25 g/L maltose + 25 g/L sucrose + 5 g/L Met + 7.5 g/L NaCl).

Table 3. Effects of in ovo injection of methionine (Met) and disaccharide (DS) on glycogen reserves of geese during the early-life period of post-hatching[1,2].

					P-value		
	Control	DS	Met	DS + Met	Met	DS	Met × DS
Pectoral glycogen contents, mg/g							
D 1 post-hatching	2.04 ± 0.22	2.45 ± 0.21	2.01 ± 0.32	2.39 ± 0.32	0.682	0.002	0.891
D 14 post-hatching	1.66 ± 0.28	1.87 ± 0.26	1.70 ± 0.31	1.78 ± 0.22	0.849	0.198	0.558
D 21 post-hatching	1.57 ± 0.18	1.66 ± 0.20	1.60 ± 0.32	1.69 ± 0.19	0.725	0.348	0.986
Liver glycogen contents, mg/g							
D 1 post-hatching	9.78 ± 0.60	11.59 ± 1.04	9.82 ± 0.78	10.13 ± 1.06	0.067	0.009	0.052
D 14 post-hatching	43.69 ± 2.36	45.21 ± 4.06	45.11 ± 2.13	43.46 ± 3.59	0.897	0.960	0.231
D 21 post-hatching	46.36 ± 4.34	43.48 ± 3.95	45.24 ± 3.86	43.55 ± 1.55	0.722	0.135	0.688

[1]The data are presented as the means ± standard deviation.
[2]Factors in four groups included noninjection (control), DS injection (25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl), Met injection (5 g/L Met + 7.5 g/L NaCl), or DS + Met injection (25 g/L maltose + 25 g/L sucrose + 5g/L Met + 7.5 g/L NaCl).

Table 4. Effects of in ovo injection of methionine (Met) and disaccharide (DS) on small intestine parameters of geese during the early-life period of post-hatching.[1,2]

					P-value		
	Control	DS	Met	DS + Met	Met	DS	Met × DS
Relative weight of small intestine, %							
D 1 post-hatching	4.16 ± 0.36	4.10 ± 0.30	5.21 ± 0.25	5.58 ± 0.19	<0.001	0.201	0.079
D 14 post-hatching	7.48 ± 0.60	7.76 ± 0.53	7.61 ± 0.37	8.07 ± 0.30	0.268	0.068	0.629
D 21 post-hatching	6.45 ± 0.59	6.25 ± 0.55	6.83 ± 0.56	6.69 ± 0.50	0.081	0.453	0.890

[1]The data are presented as the means ± standard deviation.
[2]Factors in four groups included noninjection (control), DS injection (25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl), Met injection (5 g/L Met + 7.5 g/L NaCl), or DS + Met injection (25 g/L maltose + 25 g/L sucrose + 5 g/L Met + 7.5 g/L NaCl).

Table 5. Effects of in ovo injection of methionine (Met) and disaccharide (DS) on jejunum parameters of geese during the early-life period of post-hatching[1,2].

					P-value		
	Control	DS	Met	DS + Met	Met	DS	Met × DS
Villus height of jejunum, μm							
Day 1 post-hatching	211.84 ± 13.81	244.30 ± 23.70	219.50 ± 18.74	287.43 ± 17.70	0.004	<0.001	0.032
Day 14 post-hatching	866.61 ± 72.63	911.13 ± 87.84	1,206.01 ± 41.24	1,168.30 ± 111.91	<0.001	0.921	0.236
D 21 post-hatching	1,087.45 ± 36.57	1,127.34 ± 81.41	1,229.40 ± 102.3	1,245.19 ± 69.41	<0.001	0.382	0.703
Villus width of jejunum, μm							
D 1 post-hatching	52.02 ± 7.34	55.83 ± 6.07	50.68 ± 1.22	59.41 ± 5.23	0.622	0.011	0.282
D 14 post-hatching	176.29 ± 10.86	172.42 ± 18.17	166.18 ± 12.41	163.90 ± 16.03	0.135	0.612	0.896
D 21 post-hatching	175.45 ± 17.00	176.86 ± 13.32	185.19 ± 9.44	188.75 ± 19.35	0.098	0.694	0.864
Villus surface area of jejunum, $\mu m^2 \times 10^3$							
D 1 post-hatching	10.95 ± 1.04	13.55 ± 1.07	11.13 ± 1.06	17.15 ± 2.58	0.008	<0.001	0.015
D 14 post-hatching	152.14 ± 3.96	158.05 ± 29.64	200.81 ± 21.72	190.04 ± 5.91	<0.001	0.753	0.288
D 21 post-hatching	190.75 ± 18.90	198.65 ± 9.16	228.45 ± 29.97	234.41 ± 20.15	<0.001	0.426	0.911

[1]The data are presented as the means ± standard deviation.
[2]Factors in four groups included noninjection (control), DS injection (25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl), Met injection (5 g/L Met + 7.5 g/L NaCl), or DS + Met injection (25 g/L maltose + 25 g/L sucrose + 5 g/L Met + 7.5 g/L NaCl).

injection increased jejunum sucrase activity at d 1 ($P < 0.01$) and 14 ($P < 0.05$) post-hatching, Na^+/K^+ATPase activity at d 1 post-hatching ($P < 0.01$), and alkaline phosphatase activity at d 1 post-hatching ($P < 0.05$; Table 6).

DISCUSSION

The pectoral muscle is the largest tissue for poultry, it has relatively large size and glycogen storage, plays a key role in metabolic activity (De Oliveira et al., 2008). It has been reported that feeding DS into the amnion of chicks stimulated the growth of pectoral muscle (Uni et al., 2005). Dong et al. (2013) found that the pectoral muscle of pigeons was increased by in ovo injection of DS. In the present study, in ovo injection of DS or Met increased the relative weight of pectoral muscle. Studies on the effects of embryo feeding of Met on the development of pectoral muscle were still limited, no study can be used for comparison with this study. We concluded that in ovo injection of Met or DS had

Table 6. Effects of in ovo injection of methionine (Met) and disaccharide (DS) on jejunum digestive enzymes activities of geese during the early-life period of post-hatching[1,2].

					P-value		
	Control	DS	Met	DS + Met	Met	DS	Met × DS
Sucrase activities of jejunum, $\mu mol \cdot min^{-1} \cdot g^{-1}$ tissue							
D 1 post-hatching	2.30 ± 0.18	3.32 ± 0.50	2.11 ± 0.42	2.92 ± 0.52	0.108	<0.001	0.565
D 14 post-hatching	4.70 ± 0.30	5.52 ± 0.23	4.85 ± 1.10	5.62 ± 0.81	0.678	0.012	0.945
D 21 post-hatching	5.12 ± 0.34	5.41 ± 0.35	5.47 ± 0.43	5.24 ± 0.75	0.654	0.878	0.215
Na^+/K^+ATPase activities of jejunum, $U \cdot min^{-1} \cdot g^{-1}$ tissue							
D 1 post-hatching	14.80 ± 1.39	16.92 ± 0.94	15.23 ± 1.84	17.95 ± 1.65	0.243	0.001	0.628
D 14 post-hatching	20.76 ± 2.46	21.33 ± 2.15	20.06 ± 3.22	20.15 ± 1.79	0.361	0.747	0.812
D 21 post-hatching	22.58 ± 2.14	22.62 ± 1.65	22.75 ± 2.27	21.94 ± 3.10	0.791	0.692	0.659
Alkaline phosphatase activities of jejunum, $\mu mol \cdot min^{-1} \cdot g^{-1}$ tissue							
D 1 post-hatching	6.72 ± 0.58	8.46 ± 1.19	8.54 ± 1.54	9.35 ± 1.04	0.009	0.012	0.334
D 14 post-hatching	9.84 ± 1.16	11.05 ± 0.66	10.03 ± 1.31	11.13 ± 2.30	0.826	0.071	0.923
D 21 post-hatching	12.14 ± 1.29	11.98 ± 0.53	12.10 ± 1.37	12.31 ± 1.30	0.754	0.959	0.705

[1]The data are presented as the means ± standard deviation.
[2]Factors in four groups included non-injection (control), DS injection (25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl), Met injection (5g/L Met + 7.5 g/L NaCl), or DS + Met injection (25 g/L maltose + 25 g/L sucrose + 5 g/L Met + 7.5 g/L NaCl).

positive effects on the development of pectoral muscle, which probably related to the improvement of glycogen reserves in the body (Kornasio et al., 2011).

The growth of muscle has reported to be supported by enhancing liver and pectoral muscle glycogen reserves via in ovo injection technique (Kornasio et al., 2011). Insufficient glycogen reserves will force the embryo to mobilize more muscle proteins towards gluconeogenesis, thus restricting the development of pectoral muscle (Uni et al., 2005). A sufficient glycogen supply reduced the need for glucose synthesis via gluconeogenesis from muscle protein (Bertocchi, 2019), which was benefited to the development of pectoral muscle (Uni et al., 2005). The delivery of DS into the amnion of birds' embryo has been reported to improve the embryo energy reserves status (Chen et al., 2010b). Uni et al. (2005) noted that feeding DS to the amnion of chicks increased liver and muscular glycogen storages. Foye et al. (2006) injected the DS into the embryo of turkey found an increase of liver and muscular glycogen contents. In this study, in ovo injection of DS had positive effects on the liver and muscular glycogen contents at d 1 post-hatching, which indicated that DS injection increased the glycogen store in geese during the first stage after incubation. However, Met injection had no significant effects on the glycogen reserves in geese. Therefore, we considered that the promotion of pectoral muscle development by in ovo injection of DS was related to the increase of glycogen contents in the body. In addition, the skeletal muscle as a 'demand' tissue, its development is supported by the 'support' tissue such as gastrointestinal tract (Rance et al., 2002). Therefore, a mature and well-absorbed intestine is also benefited to the development of pectoral muscle.

Early growth and development of the gastrointestinal tract are critical to optimizing the growth of poultry (Das et al., 2021). The relative weight of small intestine and the intestinal morphology are commonly used parameters to evaluate the status of gut (Chen et al., 2021). In the small intestine, the epithelium is thrown into long folds, the villi, which serve to increase the surface area for enzyme secretion and nutrient absorption (Dibner and Richards, 2004). The most villi abundant part of the small intestine is the jejunum, which leads to the jejunum are the greatest location for nutrient digestion and absorption (Vaezi et al., 2011; Liu et al., 2014). In ovo injection of DS has been reported to promote the development of villi from jejunum in poultry (Jia et al., 2011; Dong et al., 2013). Chen et al. (2010a) reported that in ovo injection of DS improved the villus surface area in the jejunum of duck. Luqman et al. (2019) observed the increase of jejunum villus surface area by DS delivery in pigeons. In the present study, the delivery of DS had positive effects on the jejunum villus height, width, and surface area, which indicated that the administration of DS promoted the development of jejunum villi. Additionally, the Met has been reported to be crucial for the maintenance of gut integrity and function (Nazem et al., 2017). In this study, in ovo injection of Met improved the development of small intestine and jejunum morphology. Similarly, Mohammadrezaei et al. (2015) reported that feeding Met into the embryo of chicks had positive effects on the absorption function by improving small intestine villus height and width. Chen et al. (2021) noted that delivery of Met increased the relative weight of small intestine and villus height in chicks. Therefore, the injection of DS or Met into the amnion of birds had positive effects on the development of the small intestine, which in turn supported the development of pectoral muscle. In addition, a synergistic effect on DS plus Met administration was observed in improving the jejunum villus height and surface area, which indicated that the injection of DS and Met is a suitable strategy for promoting the development of the small intestine.

A mature intestine is always accompanied by abundant digestive enzymes secretion and high activities (Chen et al., 2010a). The digestive enzymes play an important role in the facilitation of nutrient absorption in the host (Wang et al., 2021). Disaccharidase breaks down the DS into glucose, its high activity presents in the small intestine ensures rapid carbohydrate digestion (Shibata et al., 2019). Additionally, alkaline phosphatase is a key enzyme that involves the process of glucose absorption (Bilal et al., 2015). The increase of the amount of substrate in the intestine has been reported to increase the activity of disaccharidase (Chen et al., 2009; Dong et al., 2013). Plenty of studies have reported that the delivery of DS into the embryo of birds increased sucrase and alkaline phosphatase activity in the small intestine (Chen et al., 2010a; Luqman et al., 2019). In this study, the increase of sucrase and alkaline phosphatase were observed in ovo injection of DS. Moreover, in ovo injection of Met had positive effects on the activity of alkaline phosphatase in the jejunum. Therefore, the delivery of DS or Met had positive effects on the enhancement of digestive enzymes activities in the jejunum. In addition, the absorption of nutrients in the intestine is achieved by Na^+-dependent kinetics (Suvarna et al., 2005; Uni, 2006). Sodium transport achieves by the enterocyte's basolateral Na^+/K^+ATPase (Uni, 2006). Na+/K+ATPase pumps sodium out of cells while pumping potassium into cells, it helps maintain resting potential, affect transport, and regulates cellular volume, especially used to transport most nutrients in the intestinal tract (Kiela and Ghishan, 2016; Abbasi et al., 2018; Revajová et al., 2019). In this study, we observed that DS injection increased the activity of Na^+/K^+ATPase from jejunum, while not Met injection. Therefore, we concluded that the injection of DS had positive effects on nutrient absorption, which was achieved by increasing digestive enzymes activities and Na^+/K^+ATPase activity. In addition, Met administration was beneficial to acquire the glucose, which probably related to the improvement of jejunum morphology.

CONCLUSIONS

This study indicated that the delivery of DS into the amnion of geese embryo increased the digestive enzymes

activities and jejunum morphology, moreover, in ovo injection of Met improved jejunum digestive enzymes activities, intestinal development, and jejunum morphology. The delivery of DS plus Met synergistically improved the jejunum morphology. Improvement in intestinal development and jejunum digestive enzymes activities were beneficial to increase the uptake of nutrients through the intestinal enterocytes for distributing to other tissues such as pectoral muscle for promoting its development. Therefore, the delivery of DS plus Met is a suitable strategy for improving intestinal development during the post-hatching stage of geese.

DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

Abbasi, F., J. Liu, H. Zhang, X. Shen, and X. Luo. 2018. Effects of feeding corn naturally contaminated with aflatoxin on growth performance, apparent ileal digestibility, serum hormones levels and gene expression of Na+, K+-ATPase in ducklings. Asian-Australas. J. Anim. Sci. 31:91.

Alagawany, M., S. S. Elnesr, M. R. Farag, M. E. Abd El-Hack, R. A. Barkat, A. A. Gabr, M. A. Foda, A. E. Noreldin, A. F. Khafaga, K. El-Sabrout, and H. A. Elwan. 2021. Potential role of important nutraceuticals in poultry performance and health −a comprehensive review. Res. Vet. Sci. 137:9–29.

Association of Official Analytical Chemists. 2000. Official Methods of Analysis of AOAC International. 17th ed. Association of Office Analysis Chemistry, Arlington, VA.

Baykalir, Y., S. I. Mutlu, and Z. Erisir. 2021. The effects of in-ovo injected D-glucose monohydrate and ascorbic acid on hatchability, body weight and early post-hatch performance of geese. Eur. J. Vet. Med. 1:1–6.

Bertocchi, M. 2019. Study of the chicken physiological profile in different gut sites and upon different environmental factors. PhD Diss. University of Molise, Italy.

Bilal, T., S. Atis, and O. Keser. 2015. The effects of microbial phytase on serum calcium and phosphorus levels and alkaline phosphatase activities in broilers fed diets containing different levels of phosphorus. Acta Sci. Vet. 43:1–15.

Chen, M. J., J. Y. Zhou, Y. J. Chen, X. Q. Wang, H. C. Yan, and C. Q. Gao. 2021. The in ovo injection of methionine improves intestinal cell proliferation and differentiation in chick embryos by activating the JAK2/STAT3 signaling pathway. Anim Nutr 7:1031–1038.

Chen, W., R. Wang, H. F. Wan, X. L. Xiong, P. Peng, and J. Peng. 2009. Influence of in ovo injection of glutamine and carbohydrates on digestive organs and pectoralis muscle mass in the duck. Br. Poult. Sci. 50:436–442.

Chen, W., R. Wang, X. L. Xiong, H. F. Wan, J. Xu, and J. Peng. 2010a. Influence of in ovo injection of disaccharides, glutamine and β-hydroxy-β-methylbutyrate on the development of small intestine in duck embryos and neonates. Br. Poult. Sci. 51:592–601.

Chen, W., J. Xu, M. Tangara, and J. Peng. 2010b. Effects of in ovo injecting disaccharides and alanyl-glutamine dipeptide on the energy status in duck embryos and neonates. Anim. Reprod. Sci. 122:29–35.

Dahlqvist, A. 1984. Assay of intestinal disaccharidases. Scand. J. Clin. Lab. 44:169–172.

Dang, D. X., H. Zhou, Y. Lou, and D. Li. 2022. Effects of in ovo feeding of disaccharide and/or methionine on hatchability, growth performance, blood hematology, and serum antioxidant parameters in geese. J. Anim. Sci. 100:skac014.

Das, R., P. Mishra, and R. Jha. 2021. In ovo feeding as a tool for improving performance and gut health of poultry: a review [e-pub ahead of print]. Front. Vet. Sci. 8:754246, doi:10.3389/fvets.2021.754246, accessed August 11, 2022.

De Oliveira, J. E., Z. Uni, and P. R. Ferket. 2008. Important metabolic pathways in poultry embryos prior to hatch. Worlds Poult Sci J 64:488–499.

Dibner, J. J., and J. D. Richards. 2004. The digestive system: challenges and opportunities. J. Appl. Poult. Res. 13:86–93.

Dong, X. Y., Y. M. Wang, H. H. Song, and X. T. Zou. 2013. Effects of in ovo injection of carbohydrate solution on small intestine development in domestic pigeons (Columba livia). J. Anim. Sci. 91:3742–3749.

Elnesr, S. S., H. A. M. Elwan, Q. Q. Xu, C. Xie, X. Y. Dong, and X. T. Zou. 2019. Effects of in ovo injection of sulfur-containing amino acids on heat shock protein 70, corticosterone hormone, antioxidant indices, and lipid profile of newly hatched broiler chicks exposed to heat stress during incubation. Poult. Sci. 98:2290–2298.

Elwan, H., C. Xie, L. P. Miao, X. Dong, X. T. Zou, M. Mohany, M. M. Ahmed, S. S. Al-Rejaie, and S. S. Elnesr. 2021. Methionine alleviates aflatoxinb1-induced broiler chicks embryotoxicity through inhibition of caspase-dependent apoptosis and enhancement of cellular antioxidant status. Poult. Sci. 100:101103.

Elwan, H. A., S. S. Elnesr, Q. Xu, C. Xie, X. Dong, and X. Zou. 2019. Effects of in ovo methionine-cysteine injection on embryonic development, antioxidant status, IGF-I and tlr4 gene expression, and jejunum histomorphometry in newly hatched broiler chicks exposed to heat stress during incubation. Animals 9:25.

Felício, A. M., L. G. Gaya, J. B. S. Ferraz, C. T. Moncau, E. C. Mattos, N. P. Santos, T. Michelan Filho, J. C. C. Balieiro, and J. P. Eler. 2013. Heritability and genetic correlation estimates for performance, meat quality and quantitative skeletal muscle fiber traits in broiler. Livest. Sci. 157:81–87.

Foye, O. T., Z. Uni, and P. R. Ferket. 2006. Effect of in ovo feeding egg white protein, β-hydroxy-β-methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. Poult. Sci. 85:1185–1192.

Hassan, H. A., A. R. Arafat, K. Y. Farroh, M. S. Bahnas, I. El-Wardany, and S. S. Elnesr. 2021. Effect of in ovo copper injection on body weight, immune response, blood biochemistry and carcass traits of broiler chicks at 35 days of age [e-pub ahead of print]. Anim. Biotechnol. 1–8, doi:10.1080/10495398.2021.1874964, accessed August 11, 2022.

Jha, R., A. K. Singh, S. Yadav, J. F. D. Berrocoso, and B. Mishra. 2019. Early nutrition programming (in ovo and post-hatch feeding) as a strategy to modulate gut health of poultry. Front. Vet. Sci. 6:82.

Jia, C. L., Z. H. Wei, M. Yu, X. Q. Wang, and F. Yu. 2011. Effect of in-ovo feeding maltose on the embryo growth and intestine development of broiler chicken. Indian J Anim Sci 81:503–506.

Kadam, M. M., M. R. Barekatain, K. S. Bhanja, and P. A. Iji. 2013. Prospects of in ovo feeding and nutrient supplementation for poultry: the science and commercial applications—a review. J. Sci. Food Agric. 93:3654–3661.

Kanagaraju, P., and S. Rathnapraba. 2019. Effect of in-ovo injection of glucose and egg white protein on the production performance and gut histomorphometry of broiler chicken. Indian J. Anim. Res. 53:675–679.

Kiela, P. R., and F. K. Ghishan. 2016. Physiology of intestinal absorption and secretion. Best Pract. Res. Clin. Gastroenterol. 30:145–159.

Kornasio, R., O. Halevy, O. Kedar, and Z. Uni. 2011. Effect of in ovo feeding and its interaction with timing of first feed on glycogen reserves, muscle growth, and body weight. Poult. Sci. 90:1467–1477.

Liu, H., J. Zhang, S. Zhang, F. Yang, P. A. Thacker, G. Zhang, S. Qiao, and X. Ma. 2014. Oral administration of Lactobacillus fermentum I5007 favors intestinal development and alters the intestinal microbiota in formula-fed piglets. J. Agric. Food Chem. 62:860–866.

Luqman, Z., S. Masood, H. Zaneb, K. A. Majeed, S. Hameed, U. Ikram, S. A. Hadi, W. Haroon, M. Zeeshan, M. Farhab, and S. Gulzar. 2019. Effect of in ovo inoculation on productive performances and histo-physiological traits in commercial birds. Int. J. Sci. Eng. Res. 10:1664–1673.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217–1240.

Mohammadrezaei, H., M. Nazem, and M. Mohammadrezaei. 2015. Effect of in ovo injection of Methionine on the histomorphometry of Jejunum of chicken embryo. Biol. Forum. 7:23.

Nazem, M. N., S. M. Sajjadian, R. Kheirandish, and H. Mohammadrezaei. 2017. Histomorphometric analysis of the small intestine of broiler chick embryos injected in ovo with methionine. Anim. Prod. Sci. 59:133–139.

Nouri, S., J. G. Ghalehkandi, S. Hassanpour, and H. Aghdam-Shahryar. 2018. Effect of in ovo feeding of folic acid on subsequent growth performance and blood constituents levels in broilers. Int. J Pept. Res. Ther. 24:463–470.

Palo, P. E., J. L. Sell, F. J. Piquer, M. F. Soto-Salanova, and L. Vilaseca. 1995. Effect of early nutrient restriction on broiler chickens.: 1. performance and development of the gastrointestinal tract. Poult. Sci. 74:88–101.

Peebles, E. D. 2018. In ovo applications in poultry: a review. Poult. Sci. 97:2322–2338.

Rance, K. A., G. M. McEntee, and R. M. McDevitt. 2002. Genetic and phenotypic relationships between and within support and demand tissues in a single line of broiler chicken. Br. Poult. Sci. 43:518–527.

Reda, F. M., A. A. Swelum, E. O. Hussein, S. S. Elnesr, A. R. Alhimaidi, and M. Alagawany. 2020. Effects of varying dietary DL-methionine levels on productive and reproductive performance, egg quality, and blood biochemical parameters of quail breeders. Animals 10:1839.

Revajová, V., R. Herich, M. Levkut, R. Žitňan, E. Albrecht, M. Röntgen, M. Levkutová, V. Karaffová, Z. Ševčíková, E. Paulovičová, and M. Levkut. 2019. Immunolocalization of Na+/K+-ATPase and proliferative activity of enterocytes after administration of glucan in chickens fed T-2 toxin. Acta Vet. Brno. 87:371–377.

Saeed, M., D. Babazadeh, M. Naveed, M. Alagawany, M. E. Abd El-Hack, M. A. Arain, R. Tiwari, S. Sachan, K. Karthik, K. Dhama, and S. S. Elnesr. 2019. In ovo delivery of various biological supplements, vaccines and drugs in poultry: current knowledge. J. Sci. Food Agric. 99:3727–3739.

Shibata, M., T. Takahashi, T. Kozakai, M. Kakudo, S. Kasuga, Y. Azuma, and Y. Kurose. 2019. Active transport of glucose across the jejunal epithelium decreases with age in broiler chickens. Poult. Sci. 98:2570–2576.

Suvarna, S., V. L. Christensen, D. T. Ort, and W. J. Croom Jr. 2005. High levels of dietary carbohydrate increase glucose transport in poult intestine. Comp. Biochem. Physiol. Mol. Integr. Physiol. 141:257–263.

Tako, E., P. R. Ferket, and Z. Uni. 2004. Effects of in ovo feeding of carbohydrates and beta-hydroxy-beta-methylbutyrate on the development of chicken intestine. Poult. Sci. 83:2023–2028.

Tserveni-Goussi, A., and P. Fortomaris. 2011. Production and quality of quail, pheasant, goose and turkey eggs for uses other than human consumption. Pages 509−537 in Improving the Safety and Quality of Eggs and Egg Products. Y. Nys, M. Bain and F. V. Immerseel, eds. Woodhead Publishing, Cambridge, England.

Uni, Z. 2006. Early development of small intestinal function. Pages 28-29 in Avian Gut Function in Health and Disease. G. C. Perry, ed. World's Poultry Science Association, United Kingdom.

Uni, Z., P. R. Ferket, E. Tako, and O. Kedar. 2005. In ovo feeding improves energy status of late-term chicken embryos. Poult. Sci. 84:764–770.

Uni, Z. E., S. A. Ganot, and D. A. Sklan. 1998. Posthatch development of mucosal function in the broiler small intestine. Poult. Sci. 77:75–82.

Vaezi, G., M. Teshfam, S. Bahadoran, H. Farazyan, and S. Hosseini. 2011. Effects of different levels of lysine on small intestinal villous morphology in starter diet of broiler chickens. Glob. Vet. 7:523–526.

Wang, B., Y. Zhou, L. Tang, Z. Zeng, L. Gong, Y. Wu, and W. F. Li. 2021. Effects of Bacillus amyloliquefaciens instead of antibiotics on growth performance, intestinal health, and intestinal microbiota of broilers. Front. Vet. Sci. 8:679368, doi:10.3389/fvets.2021.679368.

Wheatly, M. G., and R. P. Henry. 1987. Branchial and antennal gland Na+/K+-dependent ATPase and carbonic anhydrase activity during salinity acclimation of the euryhaline crayfish Pacifastacus leniusculus. J. Exp. Biol. 133:73–86.

Willemsen, H., M. Debonne, Q. Swennen, N. Everaert, C. Careghi, H. Han, V. Bruggeman, K. Tona, and E. Decuypere. 2010. Delay in feed access and spread of hatch: importance of early nutrition. Worlds Poult. Sci. J. 66:177–188.

Zamani, A., F. Shariatmadari, S. Rahimi, and M. A. Karimi Torshizi. 2018. Effects of in ovo injection of carbohydrates, β-hydroxy-β-methylbutyrate, and vitamins on ostrich organ weight, bone characteristics, and small intestinal morphology. Can. J. Anim. Sci. 99:116–122.