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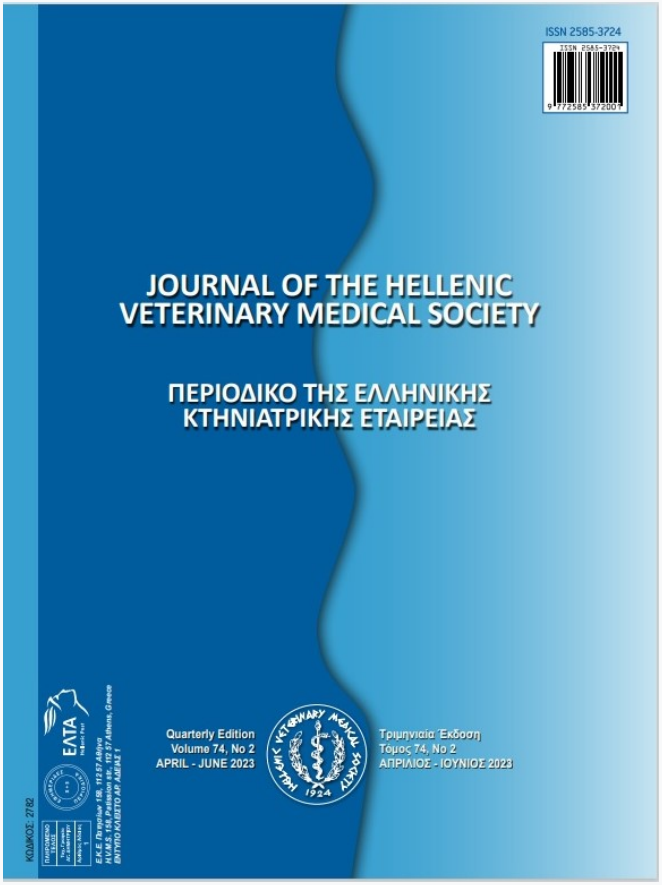

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Effects of *In-Ovo* Glucose and Glutamine Treatment on Hatching Efficiency, Intestinal Histomorphology and Gene Expression of Digestive Enzymes in Broiler Chicks

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ABSTRACT: The purpose of the present study was to identify the effects of *in-ovo* injection of glucose and glutamine on hatching efficiency, hatching weight, liver, and residual yolk weights, intestinal histomorphology and gene expressions of digestive enzymes. On the 17th day of incubation, NaCl (0.9 %), glutamine (0.5 ml 10%), glucose (0.5 ml 0.25 g/ml), and glutamine+glucose (0.25 ml 10%+0.25 ml 0.25 g/ml) were injected into the amniotic sac. *In-ovo* injection of glucose, glutamine, and glucose+glutamine significantly decreased hatching efficiency in glucose and glutamin+-glucose groups ($p<0.001$). This treatment did not affect hatching weight, liver, and residual yolk weight in any of the groups. When the intestinal histomorphology was evaluated, *in-ovo* injection was found to increase the villi height/crypt depth ratio, villus width and the number of goblet cells in the jejunum, whereas villi height, crypt depth, and tunica muscularis thickness were not significantly affected by the treatments. However, villi height of jejunum increased by approximately 16% with in ovo administration of glucose, but $p=0.052$. This may means, in ovo administration of glucose tends to increase villus height of jejunum. Moreover, the ileum histomorphology in general appear not to be affected by any of the treatments as well. *In-ovo* glutamine injection significantly increased Suc-Iso and mTOR gene expressions ($p\leq0.05$) compared to the control group, whereas SGLT1 gene expression was statistically indifferent. At the end of the study, while *in-ovo* glucose and glutamine injection had an overall negative effect on the hatching efficiency in broiler chicks.

***Keywords*:** Broiler chicks, glutamine, *in-ovo* feeding, intestinal histomorphology.

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INTRODUCTION

The perinatal period (pre-and post-hatch period) is critical in the development of the chick embryo since during this period. There is a high energy requirement for hatching and basal metabolism (Uni et al., 2005). On the other hand, towards the end of embryonal development, glucose reserves (Uni and Yahav, 2010) and various nutrient levels in egg yolk significantly decrease (Yair and Uni, 2011). As a result of these deficiencies, some adverse effects occur on the digestive system, skeletal system, and immune system, which directly affect the health and performance of the animal (Noy and Sklan, 1997). In the last days of incubation, a large amount of energy is required for the development of the digestive organs. However, the energy source for embryo development as a only is limited. Eggs are rich in protein and lipids but poor in carbohydrates. Therefore, carbohydrates found naturally in the egg are insufficient to meet the metabolic requirements of the embryo. For this reason, it may be beneficial to supplement the embryo with carbohydrates through the *in-ovo* feeding method (Tako et al., 2004; Smirnov et al., 2006; Zhai et al., 2011a).

Glutamine is a semi-essential amino acid whose deficiency only occurs under metabolic stress (Coster et al., 2003). It has many functions in the body as a nitrogen transporter, protein and nucleic acid synthesis metabolite, gluconeogenesis, and glutathione precursor, as well as an oxidative energy source (Sentongo and Mascarenhas, 2002). Glutamine is also a preferred energy source, especially for enterocytes and lymphocytes (Coster et al., 2003; Garcia et al., 2003). Additionally, it increases the resistance of the gastric mucosa and protects the gastrointestinal mucosa, protects the skeletal muscle by contributing to the nitrogen balance of the body, improves the villus functions in patients with the intestinal syndrome, accelerates the development of the mucosa, enhances muscle development and endurance, supports the immune system, increases the activity and amount of sucrase in the intestine. Many studies have shown that glutamine increases the thickness of the muscle layer and intestinal protein concentration (Calder and Yaqoob, 1999; Colker et al., 2000; Grimm and Kraus, 2001). It has also been reported that glutamine promotes intestinal development and regulates intestinal barrier function in many animals (Jacobi and Odle, 2012; Rezaei et al., 2013; Wang et al., 2014). Glutamine supplementation increases microbial metabolites, improves microbial composition and colonic development in piglets (Holthausen et al. 2022). Supplementation of glutamine can regulate the innate immune response, the intestinal mucosa barrier, and the apoptotic gene signaling pathways to provide protection against Salmonella enteritidis infections in young chickens (Wu et al. 2022). Dietary supplementation with glutamine improves the morphological development of the small intestine, the activity of intestinal mucosa disaccharides and increases the mRNA expression of ZO-1, claudin-1 and occludin proteins in broilers (Wu et al. 2020). Glutamine injection with easily soluble carbohydrates such as sucrose and maltose enable glutamine to be digested and absorbed in high amounts (Chen et al., 2009).

It is known that intestinal histomorphology and survival of chick is improved by *in-ovo* feeding treatment. Based on previously reported favourable effects of glutamine, this study was designed to investigate the effects of *in-ovo* glucose and/or glutamine treatment on hatching efficiency, intestinal histomorphology and gene expression of several digestive enzymes in broiler chicks.

MATERIALS AND METHODS

Hatching Eggs and Incubation Process

Fertilized eggs obtained from a 35-week-old breeder parent stock of 1000 Ross 308 chickens were brought to the laboratory and those were not suitable for incubation (i.e., dirty, cracked, too large, too small, deformed, etc.) were eliminated. Eggs placed in the incubation were chosen to be similar in weight, averaging 61 g. Eggs were weighed on the day of the injection -so the 17th day of the incubation- and it was approximately 56 g for all groups. After the separation process, the remaining eggs were weighed and 600 eggs with similar weights were placed again in the incubator.

The incubation process started after the eggs were disinfected by fumigation with formalin. On the 17th day of the incubation phase, from 600 eggs controlled by a lamp in a dark room, unfertilized eggs and early embryonic deaths were removed. The eggs containing 500 live embryos were divided into five equal groups, as in 100 eggs per each, as NaCl (0.9 %), glutamine (10%), glucose (0.25 g/ml) and glucose+glutamine (10% glutamine+0.25 g/ml glucose).

In-Ovo Feeding

The solutions were prepared freshly on the day of the injections. The injection volume into the amniotic fluid was set in accordance with the *in-ovo* feeding

Table 1: Solutions prepared and their osmolarity

Groups	Solutions	Osmolarity (mOsm/L)
Control	No injection	-
NaCl	%0.9 NaCl	308
Glucose*	0.25 gr/ml glucose	1696
Glutamine*	%10 glutamine	992
Glucose+glutamine*	0.25 gr/ml glucose+%10 glutamine	2688

*Solutions of these groups were made with 0.9% NaCl and injected to 0.5 ml/per egg.

procedure given by Uni and Ferket (2003) and was 0.5 ml/egg. On the 17th day of embryonic development, using sterile injectors with 21G needles, the amniotic fluid was injected from the air space of the egg. 0.9% NaCl (vol/vol) was injected solely into the eggs in the NaCl group (to determine the effect of the injection itself). 0.25 g/ml glucose was injected to the glucose group, 10% glutamine (wt/vol) to the glutamine group and 0.25 g/ml glucose+10% glutamine to the glucose+glutamine group. 0.9% NaCl was used in all dilutions in glucose, glutamine, and glucose+glutamine groups.

The injection site of the eggs before and after injection were disinfected by spraying 75% ethanol solution. All stages except shell drilling and injection were applied to the control group and errors related to manipulation were tried to be minimized. Eggs were incubated under 37.7°C temperature and 60% humidity for the first 18 days, 37°C and 70% humidity from the 18th day until the hatching day.

Determination of Hatching Efficiency and (,) Weights of Liver and Residual Yolk of the Chicks

After hatching (21st day of incubation), all the chicks were weighed individually. Subsequently, hatching efficiency was calculated by proportioning the number of hatched fertilized eggs and the number of live chicks from the hatch. Livers and yolk sacs were taken from 10 chicks of each group were also weighed. End of the weighing process chick, liver and residual yolk weights were determined.

Histomorphology Measurements

On the 21st day of incubation, the cervical dislocation procedure was applied to 10 chicks of each group and jejunum and ileum samples were taken for histomorphology analysis. The sampling process was made according to Uni et al. (2003). For jejunum, it was made in the middle of Meckel's diverticulum with the point where bile ducts are connected, while for ileum, the samples were taken from the middle of Meckel's diverticulum with ileocecal connection. The 0.5 cm intestinal dissections have taken from the jejunum and ileum were first washed with physiological saline (PS). Then that parts were fixated in 10% buffered formol for 24 hours. Afterward, it was passed through different grades of alcohol solution and xylol and blocked on the paraplast.

Dissections of 5 µm were taken from the blocks; Crosmon triple staining was done to evaluate in terms of villus height (VH) (from villi top point to the beginning of the crypt), crypt depth (CD) (from the beginning to the bottom of the crypts), villus width (VW) villus height/crypt depth ratio (VH/CD) and tunica muscularis thickness. The photographs of the sections were taken using a Leica DM 2500 brand research microscope and scaled with the help of the Leica Application Suite program. Intestinal dissections were measured by using "Image J program". During the measurement process, randomly determined 10 villi and crypts of the intestinal parts taken from the embryos were measured.

For some of the dissections, periodic acid Schiff (PAS)/alcian blue (AB) staining technique was used to reveal mucin-containing goblet cells in neutral (PAS+) and acidic (AB+) character.

The staining process was implemented following the stages proposed by Geier et al. (2011). At the end of the procedures, the cells containing neutral mucin appeared as pink (PAS+), those acidic mucin-containing ones were blue (AB+), and both neutral and acidic mucin-containing cells were purple (PAS+/AB+). For this purpose, randomly determined positive cells were counted in 10 crypt areas (mm^2) at 40X magnification.

Determination of SGLT1, Suc-Iso and mTOR Gene Expressions

In the samples taken as stated by Uni et al. (2003); SGLT1, Suc-Iso and mTOR gene expressions in the control and glutamine groups were determined by using Applied Biosystems 7500 Real-Time PCR de-

vice (ThermoFisher Scientific, Massachusetts, United States). *In-ovo* injection of glucose and glucose+glutamine appear to decrease significantly hatching efficiency. Therefore, expression profiles of SGLT1, Suc-Iso and mTOR were not determined in glucose and glucose+glutamine groups.

Gene expressions were evaluated by using quantitative real-time PCR. In real-time PCR studies, the amount of DNA amplification was measured by increasing of fluorescent light using EvaGreen dye. Primer design for SGLT-1, Suc-Iso and mTOR genes, which are used in the study, was done by using inter-racial homologies with the Perl Primer program. In addition, Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) gene was used as the housekeeping gene of the study (Livak and Schmittgen, 2001).

In real-time PCR studies, triple readings were done for each gene in all samples, the mean value of three readings for each sample was calculated and used for further estimations. Expression levels of genes were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical Analysis

SPSS 26.0 package program was used for statistical analysis of hatching efficiency and intestinal histomorphology data. Shapiro-Wilk and Levene's tests were examined to check for normal distribution assumptions and variances homogeneity. A One-Way ANOVA test was used to investigate the difference between the means of the analyzed groups. The level of significance was set to $p \leq 0.05$. Furthermore, Tukey test was implemented to compare means of treatment groups for pairwise differences.

Compared to SGLT1 and Suc-Iso gene expression data, statistical analysis was performed with independent samples T-test using the SPSS 26.0 program. Since mTOR gene expression data do not suit to a normal distribution, Mann Whitney U test was used in the statistical analysis of the data.

RESULTS

Hatching Efficiency, Chick, Liver and Residual Yolk Weights

The effects of glucose and glutamine injection on hatching efficiency are shown in Table 3. Hatching efficiency was similar in control and glutamine groups. However, glucose and glucose plus glutamine injections significantly ($p<0.001$) decreased hatching efficiency compared to the control and glutamine groups. Chick weights, liver and residual yolk weights of the hatched chicks were found to be similar in the treatment groups ($p>0.05$).

Histomorphology Measurements of Jejunum and Ileum

The effects of *in-ovo* glucose and glutamine treatment on VH, CD, VD/CD, VW, TMT and GCC in

Table 2: Primers used in quantitative real-time PCR

Target gene	Forward	Reverse
GAPDH	CCTAGGATACACAGAGGACCAGGTT	GGTGGAGGAATGGCTGTCA
SGLT1	GCCATGGCCAGGGCTTA	CAATAACCTGATCTGTGCACCAGTA
Suc-Iso	CGCAAAAGCACAGGGACAGT	TCGATACGTGGTGTGCTCAGTT
mTOR	CATGTCAGGCACTGTGTCTATTCTC	CTTTCGCCCTTGTTTCTTCACT

Table 3: Effects of in ovo glucose and glutamine injection on hatching efficiency (%), hatching, liver and residual yolk weights (g)

Treatment groups	EW (E0)	EW (E17)	HE	HW	LW	RYW
Control	61.20	56.50	93.33[a]	45.27	0.96	7.20
NaCl	61.20	56.67	94.35[a]	44.75	0.94	6.88
Glutamine	61.19	56.51	90.08[a]	44.72	0.89	6.81
Glucose	61.22	56.67	49.89[b]	44.15	0.89	6.98
Glutamine+glucose	61.20	56.63	7.81[c]	44.02	0.88	6.91
SEM	0.09	0.09	7.04	0.25	0.01	0.17
P	0.999	0.950	<0.001	0.557	0.183	0.964

EW (E0): Egg weight on the beginning of incubation, EW (E17): Egg weight on the 17th of incubation,
HE: Hatching efficiency (%), HW: Hatching weight (g), LW: Liver weight (g), RYW: Residual yolk weight (g). SEM: Standart error of the mean
a, b, c: Means with different letters in the same column are different at p<0,05 in instances with significant interaction.

jejunum and ileum were given in Table 4. The results of the NaCl group were not included because it was made only to control whether there is an effect caused by manipulation, which was not the case.

Jejunum Histomorphology

In the examination performed from the samples taken within the first 4 hours after hatching, the differences between the groups regarding VH was not found to be significant (p>0.05). With in ovo administration of glucose, villi height of jejunum increased by approximately 16%, but p=0.052. This may mean, in ovo administration of glucose tends to increase villi height of jejunum. Although there was no statistical difference, the CD was measured at the lowest in the group in which glucose and glutamine were used together and the highest in the glutamine group. Significant differences emerged between the groups regarding VH and CD ratio (p≤0.05). In the group with the greatest VH, the ratio of VH/CD was also affected, and the results were higher than that of the glutamine injected group. The group in which glutamine was given with glucose and the control group were found to be similar to all groups.

Jejunum TMT between the groups was found to be similar. There was no effect of glutamine using on the jejunum tunica muscularis thickness, which is expected to increase the thickness of tunica muscularis. GCC increased with used of glucose. The GCC measured in the glutamine group was significantly higher (p=0.001) than in the control, glucose, and glucose+-glutamine groups.

Ileum Histomorphology

No significant effect of *in-ovo* glucose and glutamine addition on ileum VH was observed. As in jejunum, the highest CD was obtained from the glutamine group. However, no difference was observed among the studied groups. The ratio of ileum VH/CD was also similar between the groups. The VW in the ileum was found to be higher in the control group, although not statistically different from the other experimental groups. Although glutamine is expected to increase the TMT of the ileum, in this study, the TMT of the ileum was found to be lowest in the glutamine group compared to the other groups. The GCC was also similar between the groups (p>0.05).

Gene Expressions of SGLT1, Suc-Iso and mTOR

Gene expressions of the digestive enzymes in those glucose and glucose+glutamine groups were not evaluated due to the negative effect of glucose injection on hatching efficiency. As seen in Table 5, SGLT1 gene expression was found to be similar to the control group in the Glutamine group. In contrast, the Suc-Iso and mTOR gene expressions increased significantly compared to the control group (p≤0.05).

Table 4: Effects of in ovo glucose and glutamine injection on small intestine histomorphology.

Jejunum						
Treatment groups	VH	CD	VH/CD	TMT	VW	GCC
Control	452.56	56.22	8.05^{ab}	112.21	74.34^{b}	19.22^{ab}
NaCl	482.98	54.99	8.78^{ab}	137.30	83.65^{ab}	19.04^{b}
Glucose	527.16	58.71	8.98^{a}	116.57	87.06^{ab}	19.06^{b}
Glutamine	465.32	60.85	7.65^{b}	106.51	76.81^{b}	19.96^{a}
Glutamine+glucose	463.06	53.44	8.67^{ab}	112.60	92.30^{a}	18.79^{b}
SEM	18.25	2.18	0.34	8.50	3.48	0.17
P	0.052	0.163	0.035	0.192	0.005	0.001
İleum						
Treatment groups	VH	CD	VH/CD	TMT	VW	GCC
Control	373.58	58.08	6.45	143.76	71.57	7.64
NaCl	365.81	55.90	6.54	131.30	61.25	7.54
Glucose	376.11	55.40	6.79	125.96	65.48	7.44
Glutamine	352.45	60.74	5.80	116.44	68.20	7.87
Glutamine+glucose	353.14	55.83	6.33	143.80	63.44	7.40
SEM	19.96	1.95	0.32	9.32	2.75	0.14
P	0.882	0.288	0.267	0.201	0.092	0.134

VH: Villus height (µm), CD: Crypt depth (µm), VH/CD: Villus height/crypt depth, TMT: Tunica muscularis thickness (µm), VW: Villus width (µm), GCC: Goblet cell count, SEM: Standart error of the mean.
a, b, ab: Means with different letters in the same column are different at p<0,05 in instances with significant interaction.

Table 5: Effects of in ovo glucose and glutamine injection on SGLT1, Suc-Iso and mTOR gene expressions.

Treatment groups	SGLT1	Suc-Iso	mTOR
Control	1.025	1.339	0.99
Glutamine	0.98	3.213	1.99
SEM	0.20	1.48	0.25
P	0.665	0.030	0.015

SGLT1: Sodium Glucose Cotransporter 1, Suc-Iso: Sucrase-Isomaltase, mTOR: Mechanistic target of rapamycin, SEM: Standart error of the mean.

DISCUSSION

In our study, hatching efficiency decreased in glucose and glucose+glutamine groups ($p<0.05$), as indicated in Table 3. The decrease seen in the glutamine group is not statistically significant ($p>0.05$). In many studies, it was seen that hatching efficiency decreases significantly with glutamine injection. In the study conducted by Youssef et al. (2017), it was determined that hatching efficiency decreased ($p\leq0.05$) with a 5 mg glutamine injection into the air sac on the 18th day of the embryonic period. Similarly, in the study conducted by Hakim et al. (2019), it was found that 0.5 ml of 1.5% glutamine injection into egg albumin on the 7th, 9th and 11th days of incubation significantly decreased the hatching efficiency ($p\leq0.05$). In the study conducted by Rufino et al. (2019), hatching efficiency increased in the group with 0.5% glutamine injection from the levels of 0.5%, 1; 1.5; 2 and 2.5 % applied directly into the air sac on the 17th day of incubation; however, it was found that hatching efficiency decreased in groups injected with glutamine at levels of 1; 1.5; 2 and 2.5%, respectively. We think that the slight decrease in hatching efficiency in the glutamine group is the osmolarity of the injected solution. Some studies conducted with glucose injection into the egg reduced hatching efficiency (Retes et al., 2018). While it has been reported that 1.0 ml injection volume does not affect hatching efficiency in general (Uni et al., 2005), Bhattacharyya et al. (2018) found that 1.0 ml glucose injection of 10% negatively affects hatching in turkeys. Hatching is usually delayed or does not occur in eggs with low water loss (Ar and Rahn, 1980; Ar, 1991) and the egg must lose 12-15% of the initial water content for a successful hatch. In the study conducted by Kanagaraju and Rathnapraba (2017), it was determined that the injection of 5 ml 5% glucose and 0.5 ml 0.4% glutamine into the amniotic sac on the 18th day of the embryonic period significantly increased the hatching efficiency ($p<0.01$) compared to the control group. In this study, it is thought that the adverse effect of glucose and glucose+glutamine injection on hatch efficiency was primarily due to dehydration caused by the high osmolarity of the injection fluid.

Pedroso et al. (2006) reported embryonic deaths from the high osmolarity of the injected solutions. Only to prevent the decrease in hatchability, when applied to amniotic fluid or chorioallantoic membrane, it has been reported that *in-ovo* injection of amino acids should be made into the extra-embryonic space or yolk sac (Ohta and Kidd, 2001). However, Campos et al. (2011) and Jia et al. (2011) state that high concentrations of carbohydrate solutions increase osmotic pressure and cause an increase in embryonic deaths. It is recommended to limit the injection volume and osmolarity to prevent excessive dehydration of the embryo and a decrease in hatchability. In our study, no difference was observed in any of the group in terms of liver weights and residual yolk weights of chicks ($p>0.05$), as indicated in Table 3. Rufino et al. (2019) found that 0.5%; 1; 1.5; 2 and 2.5 glutamine injection into the amniotic sac on the 17th day of incubation did not affect liver weight. In the study conducted by Sözcü and Ak (2020), liver weights increased in the groups in which 20, 40 and 60 mg of glutamine was injected into the amniotic sac on the 17th day of incubation. On the contrary, it decreased in the group injected with 80 mg glutamine. In the study, Sözcü and Ak (2020) found a decreased residual yolk weight in the groups injected with 20 mg, 40 mg and 60 mg glutamine, which was increased in the group injected with 80 mg glutamine ($p\leq0.05$). Rufino et al. (2019) found that glutamine injection into the air sac increased the weight of the residual yolk sac. Dos Santos et al. (2010) found that the yolk sac weight increased when they injected 0.5 ml 50% maltose into the amniotic sac on the 18th day of incubation. Furthermore, they stated that 0.5 ml of 10% glutamine solution was injected into the amniotic sac; the weight of the residual yolk sac was not affected. This weight gain was explained by using exogenous carbohydrates as a source of energy, resulting in less consumption of the embryo's yolk sac. The volume and osmolarity of the injected solution may also affect

the weight gain in the yolk sac (Retes et al., 2018). This may explain why less nutrient passage from the yolk sac to the embryo, thus increasing the values at the end of the hatch (Zhai et al., 2011b). The differences between our study and the results of the studies can be explained by the low amount of the injected molecule and the inability to evaluate glutamine as effectively as glucose in energy metabolism.

Salmanzadeh et al. (2016) and Youssef et al. (2017) stated that *in-ovo* glutamine injection increases hatching weight. Rufino et al. (2019) have noticed that hatching weight is not influenced by the glutamine injection. Kanagaraju and Rathnapraba (2017) found that the injection of 5 ml of 5% glucose and 0.5 ml of 0.4% glutamine into the amniotic sac on the 18th day of incubation significantly increases hatching weight. In our study, there was no significant difference in the hatching weights of the groups ($p>0.05$). (Table 3). The difference in the results obtained is thought to be due to the glutamine dose used. *In-ovo* glucose and glutamine treatment are considered to ineffective for hatching efficiency.

In our study, when the average jejunum VH was compared with the control group; it was determined that it increased in the experimental groups (Table 4), but the difference between the groups was not significant ($p<0.05$), and the ileum VH was not affected. Sözcü and Ak (2020) found that the jejunum VH increased after hatching with glutamine injection and that the difference between groups was significant ($p\leq0.05$). In other studies, with the addition of glutamine to rations, 0.25% (Jazideh et al., 2014) 0.5% and 1% (Abdulkarimi et al., 2019, Jazideh et al., 2014) glutamine supplementation at the end of 42 days of feeding, jejunum VH found to was increased significantly ($p\leq0.05$). Bartell and Batal (2007) stated that when 1% and 4% glutamine were added to broiler rations, the jejunum VH increased and the increase in the group with 4% glutamine was higher ($p\leq0.05$). Researchers mentioned that the effect of glutamine addition to the ration on ileum VH (Jazideh et al., 2014; Abdulkarimi et al., 2019; Gholipour et al., 2019) is not statistically significant. However, contrary to these studies, some *in-ovo* feeding studies (Sözcü and Ak, 2020) and studies where 0.5% and 1% glutamine were added to the ration (Olubodun et al., 2015) showed that the ileum VH increased significantly ($p\leq0.05$).

When the jejunum CD was compared with the control group in this study, increases were observed that were not statistically significant (Table 4). Studies in which glutamine was added to broiler diets and at the end of 42 days of feeding (Olubodun et al., 2015; Jazideh et al., 2014) reported that the jejunum CD was not affected. In other studies, it was seen that the addition of glutamine to the *in-ovo* air space (Sözcü and Ak 2020) or to rations (Abdulkarimi et al., 2019; Gholipour et al., 2019) significantly increases the jejunum CD ($p\leq0.05$). In our study, it was determined that *in-ovo* glutamine injection did not effect on the ileum CD. It was found that when glutamine was added to broiler rations, at the end of the 42 days, ileum CD increased and this increase was significant ($p\leq0.05$) (Abdulkarimi et al., 2019; Olubodun et al., 2015). Similarly, Gholipour et al. (2019) stated that glutamine added to Guinea chicken diets increased the ileum CD at the end of 42 days of feeding and that the difference between the groups was significant ($p\leq0.05$).

Increased VH is associated with increased body weight due to better nutrient absorption capacity (Caspary, 1992). However, the decrease in CD also means a decrease in the metabolic cost of the intestinal epithelial cycle (Floc'h and S`eve, 2000), which results in a better feed conversion rate. Increasing the ratio of VH/CD is essential in evaluating the effects of the feed additive product added or applied in studies (Potten, 1997; Willing and Van Kessel, 2007). In this study, this rate varied significantly between the groups. It is thought that *in-ovo* glucose treatment may have positive effects on intestinal histomorphology with appropriate dosage and proper techniques.

In this study, the GCC increased in the jejunum, but the ileum was not affected. (Table 4). While Abdulkarimi et al. (2019) reported that glutamine supplement decreased the GCC in jejunum at the end of 42 days of the feeding period, while Jazideh et al. (2014) stated in a similar study that the GCC cells increased in the jejunum, but this increase was not statistically significant. The higher level of GCC in the glutamine group, which is involved in the formation of the mucin layer that is an essential part of the nonspecific immune response in terms of intestinal health, is thought to be an essential outcome for future studies to expand the application area of this molecule. Jazideh et al., (2014), Abdulkarimi et al. (2019) and Gholipour et al. (2019) stated in their studies that the values of goblet cell numbers in the ileum do not differ significantly.

In this study, no difference was observed in the TMT of jejunum and ileum (Table 4). Sözcü and Ak

(2020) mention that glutamine injection into the egg does not affect the TMT of the jejunum and ileum. Jazideh et al. (2014) found that the glutamine added to their ration showed the thickness of the tunica muscularis after 42 days of feeding ($p \leq 0.05$).

In this study, jejunum VW was increased, while ileum VW was not affected (Table 4). Gholipour et al. (2019) reported that glutamine increases the jejunum VW ($p \leq 0.05$). However, Abdulkarimi et al. (2019) and Jazideh et al. (2014) found that VW in the jejunum is not affected by the glutamine injection ($p > 0.05$).

There is no study found on the effect of glutamine injection in eggs, despite the very little literatures examining the effect of glutamine supplementation in broiler diets on SGLT1, Suc-Iso and mTOR gene expression. The increase in mRNA expression of $b^{o,+}AT$, PepT1, and SGLT1 brush border membrane transporter on the first day of hatching in male chickens may be caused by the transport of cationic amino acids, peptides and glucose to intestinal enterocytes. SGLT1 is the primary transporter of glucose to intestinal enterocytes (Kaminski and Wong, 2018). Sucrase-isomaltase is comprises of two fully functional subunits, which are activated by the cleavage of pancreatic proteases. The sucrase subunit is responsible for the hydrolysis of sucrose and the isomaltase subunit is responsible for nearly all isomaltase activity (Semenza, 1986). The insulin signaling pathways and mTOR are extensively linked and show significant overlap. This pathway is called the insulin/mTOR signaling pathway (Punzo et al. 2008). Upregulation of these transporters may lead to an increase in the intake of critical nutrients for distribution to other tissues and organs (Kaminski and Wong, 2018). *In-ovo* injection of glutamine had no significant effect on SGLT1 gene expression in the jejunum. *In-ovo* injection of glutamine significantly increased Suc-Iso and mTOR gene expression in the jejunum ($p \leq 0.05$). (Table 5). The digestive system is not fully developed in the early weeks of life. The development of digestive functions is characterized by the development of pancreatic secretion functions and the height of brush border membrane enzymes in the intestines (Cahu and Infante, 1995; Ma et al., 2005). The reason why the addition of glutamine to the diet positively affects digestive enzymes is because that glutamine supports intestinal development, as stated by the previous studies (Yan and Qui-Zhou, 2006; Cheng et al., 2011; Cheng et al., 2012).

CONCLUSION

In conclusion, while *in-ovo* glucose and glutamine injection had an overall negative effect on the hatching efficiency in broiler chicks the application has a significant effect on intestinal histomorphology and Suc-Iso gene expression, so it has been considered to only been applicable in practices. New studies with higher levels of glutamine by considering the osmolarity of the injected solutions are recommended and these studies should be repeated for also the case of starvation stress. It is recommended to support the results with 21-42days long feeding studies.

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ETHICAL STATEMENT

All experimental procedures were approved by the Animal Ethics Committee of Ankara University (2015-18-198).

CONFLICT OF INTEREST

The authors declared no potential conflict of interest with financial support and materials used in conducting the experiment and writing this paper.

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