

# Effect of *in ovo* Feeding of Butyric Acid on Hatchability, Performance and Small Intestinal Morphology of Turkey Poults

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## Abstract

The aim of present study was to investigate effect of *in ovo* administration of butyric acid on hatchability, performance, and small intestinal morphology of turkey poults. Fertilized eggs were subjected to injections with butyric acid (10 mg, 20 mg and 30mg dissolved in 0.5 mL of deionized water) on the d 7 of incubation. Hatching traits, Body weight gain (BWG), feed intake, and feed conversion ratio (FCR) were determined during experiment. Small intestinal morphology included villus height (VH), crypt depth (CD), and villus width (VW) were measured at hatch and the end of each rearing periods. Finally, the results of the present study indicate that the weight of newly-hatched poults was significantly greater when butyric acid were administrated, in comparison with control groups. But, *in ovo* feeding (IOF) caused lower hatchability than in control group (not-injection eggs) ( $P<0.01$ ). Poults from IOF showed better weight gain and FCR (0-42 day of age), when compared to poults hatched from control ( $P<0.01$ ). The IOFB significantly increased VH for duodenum, jejunum and ileum at both hatch and starter period. It was concluded that IOFB may affect VH of intestine at hatch and starter (post-hatch) period in turkeys. Also, IOFB can improve performances.

**Keywords:** *Butyric acid, Feeding, Performance, Small intestinal morphology, Turkey poult*

## *In ovo* Bütirik Asit Beslemesinin Hindilerde Yumurtadan Çıkma, Performans ve İnce Barsak Morfolojisi Üzerine Etkileri

### Özet

Bu çalışmanın amacı *in ovo* bütirik asit beslemesinin hindilerde yumurtadan çıkma, performans ve ince barsak morfolojisi üzerine etkilerini araştırmaktır. Fertilize olmuş yumurtalara inkübasyonun 7. gününde 0.5 mL deiyonize su içerisinde çözödürölmüş 10, 20 veya 30 mg bütirik asit enjekte edildi. Yumurtadan çıkma, vücut ağırlık artışı (BWG), yem tüketimi ve yem konversiyon oranı (FCR) belirlendi. İnce barsak morfolojisini gösteren villus yüksekliği (VH), cript derinliği (CD) ve villus genişliği (VW) yumurtadan çıkma zamanında ve her bir yetiştirme periyodu sonunda ölçöldü. Çalışma bulguları kontrol grubu ile karşılaştırıldığında bütirik asit verilenlerde yumurtadan çıkma zamanında vücut ağırlıklarının anlamlı derecede büyük olduğunu gösterdi. Ancak *in ovo* besleme (IOF) kontrol grubu (enjeksiyon yapılmayan) ile karşılaştırıldığında daha düşük yumurtadan çıkmaya neden oldu ( $P<0.01$ ). *In ovo* beslenen civcivler kontrol grubu civcivleriyle karşılaştırıldığında daha iyi vücut ağırlığı kazanımı ve yem konversiyon oranı (0-42 gün) gösterdiler ( $P<0.01$ ). *In ovo* bütirik asit besleme duodenum, jejenum ve ileum villus yüksekliklerinde hem yumurtadan çıkma hem de starter döneminde anlamlı artışa neden oldu. Sonuç olarak hindilerde *in ovo* bütirik asit besleme yumurtadan çıkma ve starter dönemlerinde villus yüksekliğini etkilemektedir. Ayrıca *in ovo* bütirik asit besleme performansta iyileşmeye neden olabilir.

**Anahtar sözcükler:** *Bütirik asit, Besleme, Performans, İnce barsak morfolojisi, Hindi*

## INTRODUCTION

Early post-hatch starvation has been associated with lower satellite cell development and decreased muscle growth in starved chicks than in fed controls throughout the experiment <sup>[1]</sup>. In this study, Chicks were either fed

or starved for 48 h post-hatch (d 0-d 2, d 2-d 4 or d 4-d 6) and then refed for 41 d <sup>[1]</sup>, whereas early post-hatch feeding stimulates satellite cells and muscle growth in turkey poults <sup>[2]</sup>. Thus, Uni *et al.* <sup>[3]</sup> hypothesized that the IOF solution contained 25 g/L maltose, 25 g/L sucrose, 200 g/L dextrin, 1 g/L HMB all dissolved in 5 g/L NaCl,



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probably enhanced myoblast development during the late embryonic stages in broilers. Also, IOF of amino acid as early feeding may provide poultry companies with an alternative method for increasing the weight of newly-hatched chicken and growth performance [4,5]. Moreover, anovel method of supplementing the (IO) nutritive of oviparous species, described as feeding (IOF) within the US Patent (6592878) of Uni and Ferket [6] was demonstrated to be an effective way to administer exogenous nutrient to support the development of the embryos and neonates in poultry.

Foye *et al.*[7] reported that IOF of egg white segment,  $\beta$ -hydroxy- $\beta$ -methylbutyrate, and carbohydrates may help to overcome the constraint of limited egg nutrients in turkeys.

Overall, the organogenesis of important segments of the chick embryo are occurred at first week of incubation, in this regard, gastrointestinal organogenesis (includes foregut, midgut and hindgut) was reported at 4-7 days of incubation. In other hand, formation of most important organs includes ovary, ileum, femur, pancreas, gastrocnemius muscle, and duodenum are establish at day-9 of incubation. Because of these evidences, IOF of energy-supplemented nutrients in this critical period (day-7) can be efficient stimulator for optimal growth and development of organs [8]. In the past studies related to "in ovo feeding", almost all of the works were conducted at the days of 17-21, late-embryonic or pre-hatch stages [3,9-13], but in the present study, we evaluated the effects of IOF in early- embryonic life of turkey poult, and the IOF of nutrient supplementation in early embryonic life.

Short-chain fatty acids, such as butyric acid are considered the prime enterocytes energy source and it is necessary for the correct development of the gut-associated lymphoid tissue [14]. Butyric acid is considered as potential alternatives to antibiotic growth promoter with positive effects on gastrointestinal function and improvement feed conversion ratio [15-17]. In this regard, Pryde *et al.*[18] reported that butyric acid can be utilized as an energy source for growth and development of intestine epithelial cells in colon.

In poultry, butyric acid is considered as a selective foodstuff for optimizes growth performance during commercial rearing period [19]. The small intestine is the major site for digestion and absorption of nutrients [20-22].

In this regard, butyric acid is known as an energy source for growth and development of intestine which it was reported in Japanese quails [23].

These results suggest that butyric acid may affect the hatching traits, performance and small intestinal morphology of turkey poult by modifying the early energy status of embryogenesis. So, the main propose of the present study was to determine of effect of injection of butyric acid into turkey eggs at d 7 of incubation on

hatchability, post-hatching performance, and small intestinal morphology of turkey poult.

## MATERIAL and METHODS

### *Incubation and Injection*

Seven hundred twenty fertile eggs were collected from turkey breeder (Nicholasstrain) at 34 weeks of age. All eggs were collected from the same breeder flock, weighed and eggs with a weight of  $82 \pm 1$  g were selected for incubation (37.8°C and 63% RH). On the d 6 of incubation, the eggs were candled, and the infertile were removed. At d 7 of incubation, fertile eggs were divided into 5 treatments with 4 replicates per treatment and 36 eggs per replicate, based on completely randomized design. Treatments were includes: 1) without injection (control group), 2) *in ovo* infusion of 0.5 mL of deionized water (sham group), 3) *in ovo* infusion of butyric acid (10 mg dissolved in 0.5 mL of deionized water), 4) *in ovo* infusion of butyric acid (20 mg dissolved in 0.5 mL of deionized water), 5) *in ovo* infusion of butyric acid (30 mg dissolved in 0.5 mL of deionized water).

Then, each egg was candled to identify the location of the injection. Next, *in ovo* injection was conducted using a 22-gauge needle and 0.5 mL of IOFB solution into the yolk sac to a depth of 19 mm. The injection area on the egg shells was disinfected with an ethyl alcohol-laden swab, sealed with cellophane tape, and transferred to hatching baskets. During injection process, the control group (non-injected eggs) were removed from the incubator together with the treated groups, and kept in the same environment. Injected solutions containing butyric acid were prepared by directly dissolving butyric acid in the deionized water. Butyric acid was purchased from Silo® Co (Silo Company, Italy) and contained 25 to 30% monoglycerides in the 1 or 3 positions, 50 to 55% diglycerides in the 1 or 3 positions, and 15 to 25% triglyceride.

### *Birds*

After hatching, poult were transferred to experimental Farm of Islamic Azad University and reared for 42 days with same ration in according to NRC turkey ration [24], in according to [Table 1](#). Each treatment group and poult was identified by neck tags. All treatments were randomly assigned to 1 of 20 pens. Each pen was bedded with soft pine wood shavings and equipped with automatic drinkers, and manual self-feeders. They had *ad libitum* access to feed and drinking water. Environmental conditions of housing were constant during the trial (temperature:  $20 \pm 3^\circ\text{C}$ , RH: 60%, and 23-h lighting).

### *Data Collection*

On hatching, hatchability and hatching weight were measured. Weight of newly-hatched poult was determined

**Table 1.** Composition and nutrient contents of the basal diet distributed turkey poults for 1-21 and 22-42 days**Tablo 1.** Hindilere 1-21 ve 22-42 günler arası verilen bazal diyetin kompozisyonu ve besin içerikleri

Ingredients (%)	0- 21 day	22-42 day
Corn	45.90	33.77
Wheat	10.00	25.00
Soybean meal (48% CP)	30.91	27.90
Corn gluten meal (52% CP)	6.00	6.00
Soybean oil	4.00	3.90
Dicalcium phosphate	1.00	1.90
Limestone	1.59	1.40
Salt	0.27	0.27
VMP <sup>1</sup>	0.50	0.50
L-Lysine HCl	0.32	0.29
DL-Methionine	0.10	0.08
Calculated composition (%)		
Metabolisable energy (kcal/kg)	3102	3089
Crude Protein	22.96	22.13
Lysine	1.50	1.5
Methionine	0.49	0.48
Calcium	1.00	1.00
Available Phosphorus	0.50	0.50
ME/CP	135	139.5

<sup>1</sup> Vitamin-mineral mixture provided (per kilogram of diet): vitamin A (*all-trans-retinyl palmitate*), 8.800 IU; cholecalciferol, 3.300 IU; vitamin E (*all-rac- $\alpha$ -tocopheryl acetate*), 40 IU; menadione, 3.3 mg; thiamin, 4.0 mg; riboflavin, 8.0 mg; pantothenic acid, 15.0 mg; niacin, 50 mg; pyridoxine, 3.3 mg; choline, 600 mg; folic acid, 1.0 mg; biotin, 220 mg; vitamin B<sub>12</sub>, 12 mg; ethoxyquin, 120 mg; manganese, 70 mg; zinc, 70 mg; iron, 60 mg; copper, 10 mg; iodine, 1.0 mg; and selenium, 0.3 mg

by weighing all hatched poults. Hatchability was calculated by considering the ratio of poults hatched to the live embryos after the treatment and expressed as a percentage of fertilized eggs. In each pen, bird body weight and feed intake were recorded on d 0, 21, and 42 post-hatch. Then, mean body weight gain, feed intake, and FCR were calculated for each pen (replicate) between 0 and 21, and 22 and 42 d. In each period, BWG was calculated and expressed as grams per bird. Feed intake (g of feed intake/bird) over the entire grow-out period was calculated by totaling feed consumption in each time interval between each bird sampling.

### Morphometric Indices of the Duodenum, Jejunum, and Ileum

At hatching and the end of each rearing period, eight birds from per treatment were euthanized by cervical dislocation. Intestinal segment samples (each ~2.5 cm in length) of duodenum, jejunum, and ileum were excised and flushed with 0.9% saline to remove the contents. The intestinal segments were fixed in 10% neutral-buffered formalin. The intestinal segments collected were the loop of the duodenum, midpoint between the bile duct entry and Meckel's diverticulum (jejunum), and midway between Meckel's diverticulum and the ileo-cecal junction (ileum). The Samples were dehydrated, cleared, and paraffinembedded. Intestinal segments were sectioned at 5- $\mu$ m thickness, placed on glass slides, and processed

by hematoxylin and eosin stain for examination by light microscopy, according to Girdhar *et al.*<sup>[25]</sup>. Morphometric indices include villus height (VH) from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, villus width (VW; average of VW at one-third and two-third of the villus) were evaluated<sup>[26]</sup>. Morphometric measurements were performed on 16 villi chosen from each segment, using a table of random numbers and a computer-aided light microscope image with Openlab software (OpenlabVersion 2.2.5, Improvision, Waltham, MA<sup>[25]</sup>).

### Statistical Analysis

Results were analyzed by ANOVA using the GLM procedure of SAS software Ver. 9.1<sup>[27]</sup>. Differences between treatments were detected by the Duncan's multiple range tests following ANOVA, and values were considered statistically different at  $P < 0.05$ .

## RESULTS

The weight of newly-hatched poults in butyric acid administrated groups was significantly higher than control groups. But hatchability significantly decreased in all injected eggs in compared to the non- injection group ( $P < 0.01$ ) (Table 2).

**At Hatching:** VH of duodenum and CH of jejunum were significantly increased in birds hatched from injected eggs in compared with control groups ( $P < 0.01$ ) (Table 3).

**Starter Period (0-21 day of age):** At the end of the starter period, significantly increased VH in the duodenum, jejunum and ileum was observed for the groups with IOF of butyric acid. There was no effect of the IOFB on CH and VW in the duodenum and ileum (Table 4).

**Grower Period (22-42 day of age):** there was no significant difference in VH of duodenum between experimental groups, at the end of the grower period. No effect of injection was detected on CH and VW for

**Table 2.** Effects of IOFB on hatchability and hatching weight in turkey**Tablo 2.** Hindilerde in ovo bütirik asit beslemenin yumurtadan çıkma ve vücut ağırlığı üzerine etkileri

Treatment	Hatchability (%)	Hatch Weight (g)
Control	89.8 <sup>a</sup>	54.67 <sup>b</sup>
Sham	78.9 <sup>bc</sup>	54.72 <sup>b</sup>
Butyric acid 10 mg	80.2 <sup>bc</sup>	55.62 <sup>a</sup>
Butyric acid 20 mg	77.8 <sup>c</sup>	55.24 <sup>a</sup>
Butyric acid 30 mg	81.5 <sup>b</sup>	55.48 <sup>a</sup>
P-Value	0.0001	0.0002
SEM	0.99	0.130

<sup>a-c</sup> Averages in a column with different superscript letters are significantly different

**Table 3.** Effects of IOFB on small intestinal morphology of turkeys at hatching (d 0)

**Tablo 3.** *in ovo* bütirik asit beslemenin yumurtadan çıkma zamanında (0. gün) hindilerde ince barsak morfolojisi üzerine etkileri

Treatment	(0 d)		
	Villus Height	Villus Width	Crypt Depth
<b>Duodenum</b>			
Control	141.36 <sup>c</sup>	22.90 <sup>b</sup>	32.90
Sham	140.91 <sup>c</sup>	22.98 <sup>b</sup>	31.48
Butyric acid 10 mg	150.52 <sup>b</sup>	23.14 <sup>b</sup>	29.80
Butyric acid 20 mg	156.79 <sup>a</sup>	26.05 <sup>a</sup>	31.33
Butyric acid 30 mg	153.97 <sup>ab</sup>	25.85 <sup>a</sup>	33.35
P-Value	0.0001	0.0004	0.476
SEM	1.44	0.51	1.46
<b>Jejunum</b>			
Control	116.65 <sup>b</sup>	25.33 <sup>b</sup>	31.83 <sup>c</sup>
Sham	122.66 <sup>ab</sup>	23.20 <sup>c</sup>	33.70 <sup>b</sup>
Butyric acid 10 mg	125.31 <sup>ab</sup>	24.99 <sup>b</sup>	34.99 <sup>b</sup>
Butyric acid 20 mg	134.20 <sup>a</sup>	28.03 <sup>a</sup>	37.83 <sup>a</sup>
Butyric acid 30 mg	130.40 <sup>a</sup>	27.02 <sup>a</sup>	36.02 <sup>ab</sup>
P-Value	0.036	0.0001	0.002
SEM	3.69	0.49	0.86
<b>Ileum</b>			
Control	73.43 <sup>b</sup>	22.69	32.69
Sham	70.97 <sup>b</sup>	22.39	32.89
Butyric acid 10 mg	80.12 <sup>a</sup>	21.77	33.52
Butyric acid 20 mg	82.33 <sup>a</sup>	19.79	30.29
Butyric acid 30 mg	75.26 <sup>b</sup>	23.19	34.19
P-Value	0.0004	0.139	0.601
SEM	1.50	0.92	1.76

<sup>a-c</sup> Averages in a column with different superscript letters are significantly different

duodenum, jejunum and ileum at the end of the grower period (Table 5).

As shown in Table 6, IOFB had no significant effect on feed intake (FI) between 0 and 21, and 22 and 42 d post-hatch. To the contrary, poult from IOF of butyric acid had improved body weight gain and feed conversion ratio (FCR), when compared with the not-injected and sham controls throughout the 22-42 day of ages.

## DISCUSSION

### Hatching Traits

In the Ipek *et al.*<sup>[29]</sup> study, *in ovo* administration of glucose did not have a positive effect on hatchability of broiler chickens. On the contrary, the examination injection into the yolk sac caused significant decrease of hatchability of

**Table 4.** Effects of IOFB on small intestinal morphology of turkeys at the end of the starter period (0-21 day of age)

**Tablo 4.** *in ovo* bütirik asit beslemenin starter dönemi sonunda (0-21 günler arası) hindilerde ince barsak morfolojisi üzerine etkileri

Treatment	21 d		
	Villus Height	Villus Width	Crypt Depth
<b>Duodenum</b>			
Control	864.18 <sup>c</sup>	52.98	101.01
Sham	853.48 <sup>c</sup>	54.40	92.18
Butyric acid 10 mg	914.53 <sup>ab</sup>	52.55	91.14
Butyric acid 20 mg	898.13 <sup>b</sup>	57.08	82.99
Butyric acid 30 mg	926.47 <sup>a</sup>	56.34	87.86
P-Value	0.0001	0.199	0.373
SEM	8.43	1.52	6.19
<b>Jejunum</b>			
Control	358.93 <sup>c</sup>	52.14 <sup>b</sup>	75.53
Sham	365.97 <sup>c</sup>	49.16 <sup>b</sup>	71.88
Butyric acid 10 mg	386.89 <sup>b</sup>	52.81 <sup>ab</sup>	68.14
Butyric acid 20 mg	402.66 <sup>a</sup>	57.39 <sup>a</sup>	81.49
Butyric acid 30 mg	390.15 <sup>ab</sup>	53.34 <sup>ab</sup>	77.09
P-Value	0.0001	0.029	0.477
SEM	4.56	1.55	5.29
<b>Ileum</b>			
Control	241.13 <sup>bc</sup>	49.64	68.92
Sham	233.47 <sup>c</sup>	52.90	65.84
Butyric acid 10 mg	252.72 <sup>ab</sup>	53.05	77.14
Butyric acid 20 mg	259.41 <sup>a</sup>	50.89	75.49
Butyric acid 30 mg	262.77 <sup>a</sup>	47.57	81.61
P-Value	0.0019	0.603	0.224
SEM	4.59	2.75	5.02

<sup>a-c</sup> Averages in a column with different superscript letters are significantly different

the newly-hatched poult. Probably the decreasing rate of hatching was because of the injection into the yolk sac. Another reason by allergic cavity that is under the air sac had been causing the respiration of developing embryo to stop and die. Previous studies on IOF of hormones such as corticosteroids at embryonic d7 resulted in 35% decline of hatchability<sup>[30]</sup>. Some of reviewed reports on *in ovo* feeding especially in early embryonic life were not successful in terms of hatchability<sup>[30-34]</sup>. Also, in the present study, the IOFB into fertile turkey eggs at d 7 of incubation did not significant effect on hatchability of sham group (injected with 0.5 mL of deionized water) than other injected groups. In according to the past studies and our present observations, it seems that any IOF at early embryonic life can harmful for internal environment susceptibility and would have negative effect on hatching; this effect is largely independent from injected butyric acid (or any other feed) effect. Also, Ohta *et al.*<sup>[4]</sup> showed the effect of

**Table 5.** Effects of IOFB on small intestinal morphology of turkeys at the end of the grower period (22-42 day of age)**Tablo 5.** in ovo bütirik asit beslemenin büyüme dönemi sonunda (22-42 günler arası) hindilerde ince barsak morfolojisi üzerine etkileri

Treatment	42 d		
	Villus Height	Villus Width	Crypt Depth
<b>Duodenum</b>			
Control	756.68 <sup>ab</sup>	48.15	62.26
Sham	745.98 <sup>b</sup>	54.40	82.12
Butyric acid 10 mg	772.03 <sup>a</sup>	52.57	72.46
Butyric acid 20 mg	777.38 <sup>a</sup>	44.58	67.74
Butyric acid 30 mg	763.22 <sup>ab</sup>	59.07	76.28
P-Value	0.034	0.673	0.370
SEM	7.43	7.27	7.12
<b>Jejunum</b>			
Control	531.44	64.04	74.78
Sham	503.48	54.16	73.14
Butyric acid 10 mg	494.39	61.57	93.40
Butyric acid 20 mg	555.16	57.39	86.50
Butyric acid 30 mg	547.66	53.34	75.35
P-Value	0.144	0.613	0.250
SEM	18.83	5.60	7.23
<b>Ileum</b>			
Control	261.13	54.65	76.42
Sham	283.47	49.90	91.84
Butyric acid 10 mg	252.73	58.37	76.89
Butyric acid 20 mg	249.42	52.15	95.49
Butyric acid 30 mg	306.28	47.57	81.61
P-Value	0.174	0.931	0.166
SEM	17.67	9.22	6.38

<sup>a-b</sup> Averages in a column with different superscript letters are significantly different

amino Acid IOFB on chicken hatchability may be related to *in ovo* injection site. Leitaot *et al.*<sup>[35]</sup> investigated the effect of the IOFB into broiler breedersegs on the hatchability, reported that the IOFB may decreased the hatching rate. Adriana *et al.*<sup>[36]</sup> found that decreased hatchability was observed when chickens embryo received IOFB at d16 of incubation. Moreover, one of the important factors may affect embryo mortality is osmolality of solution, the maximum osmolality of solution was 500-600 miliosmol which suggested by Uni and Ferket<sup>[6]</sup>. Whereas osmolality of butyric acid solution in the present study was far lower than Uni and Ferket<sup>[6]</sup> recommendation.

Based on the findings of present study, the IOFB in the yolk sac can be an effective tool to increase the weight of newly-hatched poults. Also, this result generally agrees with our idea that exogenous nutrition provision can substitute for amino acids to provide energy. Thus, exogenous nutrients supply reduces the dependency of the embryo upon amino acids by improvement in embryo energy status and increases protein deposition, probably by attenuating muscle wasting to help increase the weight of newly-hatched poults. The present report confirms the earlier patent claims by Uni and Ferket<sup>[6]</sup> that IOFB enhances chick energy status and gut maturation. Also, several studies stated that IOFB can improve embryo energy status and hatch weight<sup>[3,9,28]</sup>.

### Morphometric Indices

The first barrier to nutrient metabolism in animals is the gastrointestinal tract, and its metabolic activity can have an effect on the nutrient supply of the whole animal. The nutrient utilization efficiency would be more if the nutrient loss at the gastrointestinal tract level could be minimized<sup>[37]</sup>. The integrity of the intestinal epithelium is important so as to utilize the nutrients to the maximum extent. The changes in the morphology of villi and reduction in absorptive surface area may reduce the nutrient absorption

**Table 6.** Effects of IOFB on body weight gain (BWG), food intake (FI) and feed conversion ratio (FCR) of turkey poults in starter (0-21 day of age) and grower periods (22-42 day of age)**Tablo 6.** Hindilerde in ovo bütirik asit beslemenin starter (0-21 günler) ve büyüme döneminde (22-42 günler arası) vücut ağırlık kazanımı (BWG), yem tüketimi (FI) ve yem konversiyon oranı (FCR) üzerine etkileri

Treatment	0-21 Day of Age (g)			22-42 Day of Age (g)		
	BW	FI	FCR	BW	FI	FCR
Control	674.77 <sup>b</sup>	933.80	1.383 <sup>a</sup>	1541.26 <sup>b</sup>	3081.07	1.99 <sup>a</sup>
Group sham	671.07 <sup>b</sup>	930.25	1.386 <sup>a</sup>	1550.00 <sup>b</sup>	3084.06	1.98 <sup>a</sup>
Butyric acid 10 mg	683.27 <sup>a</sup>	919.33	1.345 <sup>b</sup>	1583.30 <sup>a</sup>	3073.59	1.94 <sup>b</sup>
Butyric acid 20 mg	688.11 <sup>a</sup>	922.28	1.340 <sup>b</sup>	1577.25 <sup>a</sup>	3068.68	1.94 <sup>b</sup>
Butyric acid 30 mg	686.83 <sup>a</sup>	915.73	1.333 <sup>b</sup>	1585.02 <sup>a</sup>	3070.63	1.93 <sup>b</sup>
P-Value	0.0001	0.524	0.006	0.0001	0.455	0.0001
SEM	1.76	8.26	0.01	3.14	6.79	0.005

<sup>a-b</sup> Averages in a column with different superscript letters are significantly different

and hence lead to reduced production performance. Considering the effect of butyric acid to improve the intestinal morphology and increase performance. In the present study, it was observed that the IOFB can increase growth in gastrointestinal tract and improve performance of poult.

As already reported by other authors [14,16,17,23,38-40] butyric acid is the major development promoter of the gastrointestinal tract, as confirmed by our results about increase growth of gut.

Additionally, preliminary studies demonstrated that IOF significantly increased intestinal villus width, goblet cell density of jejunum villi and surface area in comparison with the controls in broiler chicks at hatch [28,41-43]. Also, Chen *et al.* [11] demonstrated that the IOF of glutamine and carbohydrates into duck eggs increased intestine weight.

### Performances

Several authors concluded that IOF improved post-hatch growth [3,7,28]. These results are in agreement with that of Zhonghong and Yuming [44] who observed that the dietary sodium butyrate supplementation at the level of 500 mg/kg increased body weight gain from 0 to 21 days and improved FCR during the period from 0 to 42 days. Also, Antongiovanni *et al.* [38] reported positive beneficial effects of BA on production performance traits of broiler chickens. To the contrary, in another study Leeson *et al.* [16] stated that the dietary butyric acid had no effect on weight gain in starter, grower and finisher periods. But, birds consumed less starter feed when diets were supplemented with butyric acid relative to the control birds.

These results indicated that IOFB can contribute to cumulative effects of butyric acid on the morphology of the gastrointestinal tract, which may cause an increase in the absorption of the nutrients from the gut and improve performance. Also, to reduce the utilize liver glycogen reserves and the depletion of muscle protein, we hypothesized that the IOFB into the yolk sac prior to hatching would support the energy status of the hatching by elevating the glycogen reserves, moderating the use of muscle proteins, and thus contributing to enhanced post-hatch performance. As a result of these properties of butyric acid, IOFB may increase growth in the gastrointestinal tract and can improve small intestinal morphology and performance. Tako *et al.* [28] demonstrated that body weight of *in ovo* fed-chicks were significantly greater than the controls at hatch through 10 days post-hatch. Also, Foye *et al.* [7] stated that IOF of egg white protein,  $\beta$ -hydroxy- $\beta$ -methylbutyrate, and carbohydrates can elevates 5 to 6% of turkey body weight.

In conclusion, results of the present study showed that the IOFB may improve development of the gastrointestinal tract and consequently increase the growth performance of turkey poults. Thus, this result generally agrees with

our hypothesis that IOFB may provide energy for small intestine and poult embryo activity, in turn alleviating energy lack, sparing the pectoral muscle protein and increasing breast muscle mass and consequently, increase the growth performance. Finally, *in ovo* feeding of butyric acid can be suggested as a method for optimizing final weight in poultry farming. Overall, these results are in agreement with the finding of Salmanzadeh *et al.* [45] who, reported that, *in ovo* administration of L-carnitine into turkey eggs significantly improve hatch weight, growth performance (0-21 and 22-42 day of age) and carcass characteristics (42 day of age) whereas hatchability significantly depressed in all injected eggs compared to the not injected ones.

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