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ORIGINAL ARTICLE

The effects of *in ovo* Injection of Butyric acid into Quails Breeder eggs on Hatchability, Growth Performance, Development of the Gastrointestinal Tract, and Carcass Traits of Japanese Quails

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ABSTRACT
The current research was designed to determine the effect of in ovo injection of butyric acid into Japanese quails breeder eggs on hatchability, growth performance, development of the gastrointestinal tract, and carcass traits of Japanese quails. On the 5th of incubation, 1200 fertile eggs, on based completely randomized design were allotted to sex treatments with four replicates per treatment and 50 eggs per replicate. Fertilized eggs were injected into the yolk sac with butyric acid (15, 25, 35 or 45 mg dissolved in 0.1 ml of deionized water) at the 6th day of incubation. Two control eggs groups (not injected and injected with 0.1 ml of deionized water) were also included. Hatching traits, performance, morphometric indices of intestine (villus height and villus width) and, carcass traits (carcass weight and relative weights of breast, liver and gizzard) on day 21 and 42 post-hatching were assessed. Hatchability was significantly increased in eggs injected with butyric acid compared to the control groups. Moreover, weight of newly-hatched quails, weight gains, villus height, villus width were significantly increased in chicken from butyric acid injected eggs compared to the control groups. In addition, carcass weight and relative weights of breast and liver were also significantly increased in quail's treated in ovo injection with butyric acid. In conclusion, the in ovo of butyric acid may improve development of the gastrointestinal tract and consequently increase performance of Japanese quails.
***Key-words**: butyric acid, in ovo injection, hatchability, performance, intestine, quail*

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INTRODUCTION

Fireman *et al.* [1] in a study acknowledge butyric acid is considered the prime source to enterocytes and it is also necessary for the correct development of the gut associated lymphoid tissue. On the other hand, during the early days post hatch, the gut undergoes rapid morphological, biological, and cellular development to better digest and absorb orally consumed nutrients [2, 3]. However, newly-hatched quails may experience suboptimal nutrient digestion and absorption during this window of time in which the gut is reaching functional maturity. It has been hypothesized that low digestive and absorptive capacity of the gut [4] may limit the energy and nutrients to the chick [5, 6], and delay subsequent early growth of newly hatched chickens. We hypothesize that in ovo injection of butyric acid into quails breeder hen eggs may provide energy for development of the gastrointestinal tract to improve nutrient absorption and quail embryo activity and consequently increase growth performance and carcass weight of Japanese quails. Together with supplying energy, the addition of butyric acid to quails eggs improves the absorption of fat- soluble vitamins, increases the efficiency of utilization of energy. Thus, the aim of the present study was to test the effects of in ovo injection of butyric acid in development of the gastrointestinal tract, growth performance and carcass traits in japanese quails.

METHOD AND MATERIALS

Incubation and in ovo injection

In this experiment, 1200 fertile eggs obtained at 5 days of incubation from a commercial hatchery, and incubated rate of humour (37.7°C and %67 RH) according to standard hatchery practices. Fertile eggs, on based completely randomized design were allotted to 6 treatment groups with 4 replicates per treatment groups and 50 eggs per replicate. At 6 days of incubation, each egg was candled to identify the location of the injection. A hole was incised using a 19 gauge needle, and 0.1 ml of in ovo feeding solution was injected into the yolk sac. The injection sited was disinfected with ethyl alcohol, sealed with cellophane tape and transferred to hatching. The in ovo injection solutions were the following: 1) non - injection (control group); 2) 0.1 ml of deionized water; 3) butyric acid 15 mg dissolved in 0.1 ml of deionized water; 4) butyric acid 25 mg dissolved in 0.1 ml of deionized water; 5)butyric acid 35 mg dissolved in 0.1 ml of deionized water; 6) butyric acid 45 mg dissolved in 0.1 ml of deionized water. Non injection group was subjected to the same handling procedures as the IOF treatment groups. Butyric acid was supplied from silo® Co of Italy and contained 25 to 30% mono glycerides in 1 or 3 positions, 50 to 55% diglycerides in 1 or 3 position, and 15 to 25% triglyceride. The products of pH and asmolarity were 6.1 to 7.8 and 70 to 147osm, respectively. All of the treatment solutions were prepared in outoclaved water.

Birds

Immediately after hatching, quails were transferred to the experimental farm of Khoy Islamic Azad University and reared for 42 days with some ration according to standard quail ration (National Research Council, 1994) [7]. All quails and treatment groups were randomly allotted to 1 of 24 cages. Each cage was equipped with automatic drinkers and self feeders. Quails had ad libitum access to feed and water. Environmental conditions of housing were constant during the trial: temperature of 20±3 °C, RH of 60% and 23-h photoperiod. All experimental protocols were approved by the Institutional Animal care and use committee at Khoy Islamic Azad University.

Data collection, Morphometric Indices and Carcass Traits

In each cage, total quail body weight, quail numbers and the weight of unconsumed and added feed were recorded on days 0, 21 and 42. Mean body weight gains, feed consumption and feed conversion ratios were calculated for each cage (replicate) between 0 and 21 and 22 and 42 days. For each time period, body weight gain was calculated and expressed as grams per bird. Food intake (g of food intake/bird) over the entire grow-out period was calculated by totalling food consumption in each time interval between each bird sampling. Food conversion ratio (g of food intake /g of body weight gain) was calculated by dividing total food intake by total weight gain in each cage.

On day 42, 12 quails per treatment were randomly chosen for the determination of organ weights and morphometric indices of intestine that includes villus height and villus width. For that, quails were weighted and slaughtered by cervical dislocation then the abdominal cavity was opened. The weight of carcass, breast, liver and gizzard were recorded and the corresponding percentages (% of live body weight) were calculated.

Then, the intestine was removed, and tissue samples from jejunum were taken for morphometric indices evaluation that included villus height (VH) from the tip of the villus to the crypt, and villus width (VW; average of VW at one-third and two-third of the villus) [8]. To measure villus height and width, 2 cm segments from the middle part of the jejunum were removed, flushed with physiological saline and immediately put into a 10% buffered formalin solution until further processing. After embedding the samples in paraffin, 5 μm sections of each sample were placed on a glass slide and then stained, using haematoxylin and eosin. A total of 10 villi per sample (40 villi per treatment) were measured using light microscope [9].

Statistical analysis

Results were subjected to procedures for completely randomized designs using the general linear model (GLM) procedure of the SAS program (SAS Institute, 2003) [10]. Differences between treatments were compared by the Duncan's multiple range tests following ANOVA, and values were considered statistically different at $p < 0.05$. When data were percentages they were transformed by arc sin square root.

RESULTS AND DISCUSSION

In the past studies on *in ovo* injection, almost all of the works were conducted on duck, broiler, white leghorin and turkey. The novel point of the present study was to evaluate the effects of *in ovo* injection in early- embryonic life of quails, especially by in ovo injection of material nutrient supplements that can be useful during the whole embryonic life of the bird (until hatching).

In ovo injection of butyric acid (15, 25, 35 or 45) mg dissolved in 0.1 ml of deionized water at 6 days of incubation of quail's breeder eggs caused significant increase of hatchability and weight of newly-hatched quails in the present study (Table 1). On the contrary, Zhai*et al.* [11, 12] demonstrate that in ovo injection into fertile single comb white leghorn and broiler breeder eggs at days 17- 18, the late- embryonic or pre-hatch stages had no significant effect on the hatchability. Also, Salmanzadeh*et al*. [13, 14] stated that hatchability was slightly but significantly depressed in all injected eggs compared to the not injected ones, but significantly increased in eggs treated with L-carnitine compared to the sham egg controls. Also, Salmanzadeh*et al.* [13, 14] demonstrated that, weight of newly-hatched poults was significantly higher when L-carnitine was received than sham and control group. Based on the results of present study, the in ovo injection of butyric acid in the yolk sac can be seen as an effective tool to increase the weight of newly-hatched quails. Furthermore, these weight advantages were sustained through to the end of the experiments at 42 days of age. Numerous studies have stated that the weight of newly-hatched chickens is a major predictor of marketing weight in chickens. Wilson [15] indicated that each 1 g of increase in weight of newly- hatched chickens leads to 8 to 13 g of increase in body weight. In this research I showed that a 5g difference in weight of newly-hatched quails due to in ovo injection of butyric acid resulted in 8 g of increase in BW at 42 days of age.

As shown in Table 2, there were no significant treatment effects on feed intake throughout the experimental period. But, quails from in ovo injection of butyric acid showed improved body weight gain and feed conversion ratio compared to quails hatched from sham and control group between 0 and 21, 22 and 42 post hatch. Salmanzadeh*et al.* [16] stated that Weight gains were markedly increased in quails supplemented with butyric acid compared to the control quails during the starting (day 0 to day 21) and the growing (day 22 to day 42) periods. Although the differences were not statistically significant, it was also noted that the food intake numerically decreased in treated birds compared to the not supplemented quails. Consequently, food efficiency was significantly improved in butyric acid supplemented quails for the both periods. Zhonghong and Yuming [17] observed that the dietary sodium butyrate supplementation at 500 mg/kg increased body weight gain from 0 to 21 days in agreement with the present results. Also, Antongiovanni*et al*. [18] reported positive beneficial effects of butyric acid on production performance traits of broiler chickens. However, Leeson*et al.* [19] have not found any effect on weight gain during the starting, growing or finishing periods in broilers supplemented with butyric acid.

As showed in Table 3, the carcass weight and relative weights of breast and liver were significantly augmented in quails injected in ovo by butyric acid compared to the control (not injected and sham) groups whereas the gizzard proportions were not affected. Thus, in ovo injection of butyric acid as additional energy source probably supported the development of the embryo, resulting in a significant increase in weight of newly-hatched quails, body weight gains and breast weight. Leeson*et al*. [19] stated that carcass weight and breast meat yield significantly increased in birds fed with 0.2% butyric acid supplemented diet. In agreement with that, Salmanzadeh*et al.*[16] reported that, the carcass weights and the relative weights of breast were also markedly increased in 42 days old quails fed with butyric acid supplemented diets compared to the controls whereas liver, heart and gizzard relative weights were not significantly altered. In the experiment using in ovo injection of butyric acid resulted in ovo increase of approximately 1 g of breast muscle.

It was observed that the villus height and villus width were significantly increased in quails injected with butyric acid compared to control groups. Salmanzadeh*et al.*[16] reported that, the villus dimensions (height and width) were dramatically increased in quails supplemented with butyric acid since the end of the starting period (on day 21) ($p < 0.01$ and $p < 0.001$, respectively) and this effect in treated quails was also marked when birds were slaughtered at the end of the experimental period (day 42). On the contrary, Vieira *et al.* [20] indicated that the addition of a blend of organic (lactic, acetic, phosphoric, and butyric) acids did not significantly affect villus height either on days 7, 14, 21 or 42 in broilers.

Increased villus height provides a greater surface area for nutrients absorption and consequently, higher performance [21]. On the contrary, reduction in villus height can reduce nutrient absorption due to the decrease in the intestinal surface area for absorption. Thus, reduction in nutrient absorption, decreased resistance to disease and lower growth performance and increase in secretion of gastrointestinal tract are the negative consequences of deeper crypt and shorter villi [22].

These data suggest that the in ovo injection of butyric acid improve development of the gastrointestinal tract and consequently increase performance of Japanese quails.

Table 1: Effect of *in ovo* injection of butyric acid on quail weight and percent hatchability in newly-hatched quails.

Treatments	**Hatchability (%)**	**Hatch Weight (g)**
Non injection	86.72^{b}	6.34^{b}
Sham group	86.01^{b}	6.22^{b}
Butyric acid 15 mg	91.04^{a}	6.72^{a}
Butyric acid 25 mg	92.32^{a}	6.69^{a}
Butyric acid 30 mg	93.72^{a}	6.90^{a}
Butyric acid 35 mg	90.89^{a}	9.83^{a}
SEM	1.39	0.05
P-Value	0.0001	0.0001

$^{a-b}$ Averages in a column with different superscript letters are significantly different

Table 2: Effect of in ovo injection of butyric acid on body weight gain, feed intake and food conversion ratio of quails in different period

Treatments	**Starting period (day 1- day21)**			**Growing period (day 22- day 42)**		
	BWG	FI	FCR	BWG	FI	FCR
Non injection	97.47^{b}	269.23	2.76^{a}	114.23^{b}	553.42	4.84^{a}
Sham group	96.72^{b}	263.41	2.72^{a}	112.71^{b}	550.17	4.88^{a}
Butyric acid 15 mg	102.59^{a}	254.73	2.48^{b}	121.42^{a}	549.81	4.52^{b}
Butyric acid 25 mg	104.78^{a}	259.92	2.48^{b}	120.72^{a}	550.64	4.56^{b}
Butyric acid 35 mg	106.29^{a}	261.03	2.45^{b}	123.51^{a}	547.23	4.43^{b}
Butyric acid 45 mg	104.21^{a}	263.12	2.52^{b}	120.89^{a}	549.23	4.54^{b}
SEM	1.08	2.64	0.046	1.09	1.71	0.05
P-Value	0.0001	NS	0.0001	0.0001	NS	0.0001

$^{a-b}$ Averages in a column with different superscript letters are significantly different

Table 3: Effect of *in ovo* injection of butyric acid on carcass, breast, liver and gizzard of quails

Treatments	**Carcass (%)**	**Breast (%)**	**Liver (%)**	**Gizzard (%)**
Non injection	66.01^{b}	22.92^{b}	2.12^{b}	1.14
Sham group	66.12^{b}	23.01^{b}	2.14^{b}	1.12
Butyric acid 15 mg	67.11^{a}	23.81^{a}	2.35^{a}	1.13
Butyric acid 25 mg	66.89^{a}	23.52^{a}	2.29^{a}	1.10
Butyric acid 35 mg	66.92^{a}	23.49^{a}	2.32^{a}	1.14
Butyric acid 45 mg	97.04^{a}	23.74^{a}	2.36^{a}	1.12
SEM	0.126	0.049	0.046	0.041
P-Value	0.0001	0.0001	0.0001	NS

$^{a-b}$ Averages in a column with different superscript letters are significantly different

Table 4: Effect of in ovo injection of butyric acid on Villus height and Villus widthof quails in different period

Treatments	**Villus height (μm)**		**Villus width (μm)**	
	(day 21)	**(day42)**	**(day 21)**	**(day42)**
Non injection	72.52^{b}	252.22^{b}	14.47^{b}	51.06^{b}
Sham group	71.92^{b}	254.78^{b}	14.23^{b}	52.16^{b}
Butyric acid 15 mg	79.42^{a}	267.23^{a}	15.74^{a}	54.91^{a}
Butyric acid 25 mg	80.31^{a}	271.47^{a}	16.01^{a}	55.72^{a}
Butyric acid 35 mg	82.54^{a}	269.18^{a}	15.92^{a}	58.42^{a}
Butyric acid 45 mg	81.92^{a}	272.19^{a}	16.12^{a}	55.32^{a}
SEM	1.50	2.51	0.52	1.06
P-Value	0.0001	0.0001	0.0001	0.0001

$^{a-b}$ Averages in a column with different superscript letters are significantly different

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