Global Veterinaria 7 (5): 468-477, 2011
ISSN 1992-6197

Effects of *in ovo* Injection of Butyric Acid in Broiler Breeder Eggs on Hatching Parameters, Chick Quality and Performance

[1]*Ahmad Salahi,* [1]*Seyed Naser Mousavi,* [1]*Farhad Foroudi,*
[2]*Mozhdeh Moosanezhad Khabisi and* [3]*Mojtaba Norozi*

[1]Department of Animal Science, Islamic Azad University, Varamin-Pishva Branch, Varamin, Iran
[2]Department of Animal Science, Islamic Azad University, Kahnooj Branch, Kerman, Iran
[3]Mahan Chicken Meat Complex, Tehran, Iran

Abstract: The experiment was conducted to evaluate the effect of butyric acid glyceride injection into amniotic fluid on hatching, chick quality, organs weight and performance parameters broiler breeder chick. From flock with 45 week age, 504 Ross 308 eggs with 66.5±0.5 g, in a completely randomized design with 3 treatment (including control or without injection, injection of Nacl 0.9% or positive control and 0.3% butyric acid injection), 4 replicate and 42 eggs on each replicate were selected. The results showed that butyric acid injection on chick weight at hatch day was not significant but chick yield was significant ($P<0.05$). Also there were significant differences in chick length and incubation duration ($P<0.05$), but there were not significant effects on hatchability and chick quality. Chick body weight at 10 day post hatch was effected by butyric acid injection ($P<0.05$) and butyric acid injection caused to increased 7.1 g chick weight on 10 day compare with control. Effect of butyric acid injection on small intestine and jejunum length on hatch day and yolk free body mass (YFBM) was significant ($P<0.05$) and butyric acid injected treatment had longer small intestine and jejunum length compare with control. Injection effect on breast weight was not significant, but on thigh weight, liver and heart weight at 7 day post hatch was significant ($P<0.05$). Duodenum weight at hatch day was affected by injection ($P<0.05$). We can conclude that butyric acid injection on chick weight at hatch day was not significant but this effect on 10 day was significant, also increased small intestine length and this topic help to increase consumption, absorption of feeds and increased body weight.

Key words: *In ovo* injection · Butyric acid · Chick quality · Incubation parameters · Broiler breeder eggs

INTRODUCTION

Butyric acid is a medium-chain fatty acid with four carbons (C4), generally absorbed from the first part of gastrointestinal [1]. Butyric acid had dual effects on bacterial (antibacterial) control and promotion of intestine villi [1, 2]. It is believed that dietary supplement of butyric, propionic and acetic acid increases the production rate of crypts cells in the rat intestinal villi [3]. Prydw *et al.* [4] reported that butyric acid is used as an energy source for growth and development of intestine epithelial cells [4] and increased ileum villi length in pigs [5]. Dietary supplementation of butyric acid improved broiler performance and carcass parameters [6] and reduced the expression of genes involved in Salmonella invasion at the lower doses [7]. Mahdavi and Torki [8] showed that dietary addition of 3g/kg butyric acid improved intestine length in compare with control group [8]. The positive effect of butyric acid was not observed in chickens raised under clean environment [8]. Also Mono butyric acid glycerides were reduced mortality rate in birds infected with *Eimeria spp* strains, but had no effect on the normal and non-infected birds [9, 10].

Antongiovanni *et al.* [11] reported that dietary addition of mixed triglyceride of butyric acid (mono, 2 and triglycerides), increased liver weight at slaughter and could be improved feed conversion ratio. However addition of 0.2% of this mixture to broiler diets, decreased villi length and increased microvilli length and crypt depth of jejunum [11].

Corresponding Author: Ahmad Salahi, Department of Animal Science, Agriculture School, Islamic Azad University, Varamin-Pishva Branch, Varamin, Tehran, Iran.
Mob: +98-9131434146, Fax: +98-2922034888, Email: ahmad.salahi2010@gmail.com.

It is claimed that, application of butyric acid glycerides in broiler diets could to improve palatability. Due to the incomplete establishment of gastrointestinal (GI) microbes in the early post-hatch, there is not adequate production of butyric acid in the intestine of chickens [2]. Van der Wielen *et al.* [12] reported that short-chain fatty acids level in chicken caecum and intestine is very low at early period of life and application of butyric acid supplements in the ration could be a suitable select for newborn animals [6]. Use of *in ovo* feeding could improve the nutritional status of the embryo and hatched chicken. Therefore, it was proposed that *in ovo* administration of butyric acid during last days of incubation could improved hatching parameters, chick quality and performance.

MATERIALS AND METHODS

Experimental Groups: Total of 504 fertile eggs were obtained from a 45 Wk breeder flock (Ross 308) and then allocated to three treatment groups (control or non-injected, *in ovo* injection of Nacl 0.9% solution or positive control and *in ovo* injection of 0.3% butyric acid solution), in 4 replicate and 42 eggs per replicate. Mean weight ± SD of selected eggs were 66.5±0.5 g.

Butyric Acid Solution: Butyric acid solution used in the experiment was Monobutyrin-Hydro C4 30 (HYDRO C4-30) liquid, water soluble product. The Hydro C4 composition assigned by manufacturer was, 47-53% butyric acid glycerides, 42 to 46 percent free glycerol, 28 to 32 percent butyric acid and 0.5-1% moisture. Also the products pH and osmolarity was 6 to 7 and 0.017 to 0.028 mol/ L (from 0.2% to 0.33% solution), respectively [13].

Based on the manufacturer recommendations if the product is used as an energy source due to butyric acid in drinking water should be mixed for poultry and calves. The recommended level of Hydro C4 in broiler drinking water was 0.2 to 0.33% from 0 to 21 days of age [13].

***In ovo* Injection Prodecure:** Prior to injection, all eggs were candled for examination of embryo position, amniotic fluid and embryo movement also a coumassie blue dye solution was injected into the 504 eggs to ensure that the solution is administrated into amniotic fluid.

After examination the eggs were *in ovo* injected at 453 h (18.8 d) of incubation using 22 needle-guage. Solution volume for each injection was 1 ml [14] and needle depth was 2.54 cm (1 inch) [15]. After injection, site of injection was sanitized with ethanol 70% [16] and injection site was sealed with a liquid adhesive.

Incubator and Incubation Duration: After 6 hours of injection, eggs transferred to hatcher. Eggs were set in hatching trays horizontally. Trays were covered by mesh wire to avoid of mixing of adjacent compartment chicks the hatcher (Petersime model 192, analog) temperature and relative humidity was 37.5 °C and 50%, respectively. Between 472 to 510 h of incubation, the hatched chicks were recorded every 2 hours [17]. Spread of hatch (hatch window) was assessed relative to the time of taking the chicks out of the hatcher [18]. Average incubation time of each replicate was calculated by number of hatched chicks× hours of incubation and the result divided by number of hatched chicks.

Hatchability and Chick Quality: Chicks were taken-off from hatcher at 508 hours of incubation when 5% of chicks were still damp at the back of the neck [18, 19]. The chicks were counted and then chicks were weighed individually. Normal chicks without defects separated and number of this chicks divided by eggs set in the incubator× 100 equal saleable chick percentage (saleable hatchability %). Second class or non saleable chick (with defects and malformation) were separated and counted. Divided by the number of non saleable chicks to eggs sett × 100 equal second class chicks percentage. Sum of saleable and non saleable chick percentage was obtained as a total hatchability percentage. Chicks were kept in chick room with 23 °C and 65 to 70% relative humidity for 2 hours, then chick quality including individual body weight, chick length and Tona score was assessed [20]. Chicks were individually weighed and chick length was measured from the tip of beak to the toe [20, 21]. For Tona score measurement chicks were scored according activity, down and appearance, eye (s), Legs, navel status, retracted yolk, navel area, remaining membrane and remaining yolk [22].

Organ Weight Measurment: Upon hatch, 2 chicks were randomly selected and euthanized by carbon dioxide (CO2) asphyxiation. The weight of wet yolk sac [19], small intestine, liver, heart, breast, thigh (s) was determined and their percentage was calculated on the basis of yolk free body mass (YFBM) and live body weight. The gut samples were taken from the following 3 sites: duodenum (from gizzard until duodenal loop), jejunum (from the duodenal loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to ileo-ceacal junction) [23].

Birds Housing: After hatch, hatched chicks were transfer to rearing room and raise on floor pens until 10 days of age separate confinement. Birds of each pen had

Table 1: Ingredients and chemical analysis composition of the starter diets

Ingredients	Unit	
Metabolizable energy	Kcal/Kg	3100
Crude protein	%	22
Crude fat	%	6
Lysine	%	1.4
Methionine	%	0.64
Methionine + cystine	%	1
Available phosphorus	%	0.45
Calcium	%	1
Sodium	%	0.2

ad libitum access to feed and water. Chicks were raised under similar environmental conditions based on broiler breeder recommendation [24]. Chicks were allocated to pens at 10 /m^2 stocking density. Light intensity was maintained 30-40 Lux and 23L:1D h photo schedule was applied throughout the experiment. No vaccine or drug antibiotics were used during 10 days rearing period. All birds had access to similar diet. The nutrient composition of diet is presented in table 1. Subsequently, feed conversion ratios (FCR), body weight gain (BWG) were calculated. Two chicks selected from each pen and fasted for 8 hours and were sacrificed at 3, 7 and 10 days. Carcass traits including carcass yield, breast muscles, thigh muscles, liver, heart and small intestine weight were determined.

Data Analysis: Data were analyzed by the GLM procedure of SAS [25] in a completely randomized design. Mean values were compared using Duncan multiple ranges. Statistical model used for data analysis was as follows:

$$Yij = \mu + Ti + Eij$$

Yij = value-per-view (observation)
μ= Average (Mean of observation)
Ti = effect of treatment
Eij = residual effect (experimental error)
Statistical significant was considered at P = 0.05.

RESULTS

Effect of *in ovo* feeding of butyric acid on hatchability, hatchling body weight, chick quality, was not significant (Table 2). Chick yield, chick length and incubation duration was affected by butyric acid injection ($p< 0.05$). Maximum chick length was observed in butyric acid injection treatment. Also the highest of incubation duration related to control group. Effect of *in ovo* injection of butyric acid solution on chicks body weight at 1, 3 and 7 days post-hatch and feed conversion ratio at 0-3 and 0-10 days was not significant. But effects of injection on body weight at 10 days and feed conversion ratio at 0-7 days was significant ($p< 0.05$). Chicks hatched from eggs injected with butyric acid solution had 2.38 gr higher body weight and 4.2% improved feed conversion ratio at 0-10 days compared to control group (Table 3).

Table 2: Effects of *in ovo* injection of butyric acid on hatchability and chick quality

		Chick Yield						
		----------	----------	chick quality		Sale	chick	incubation
Treat	Chick wt (g)	To Egg wt sett (%)	To Egg wt candle (%)	Score	hatchability (%)	hatch (%)	length (cm)	duration(h)
1 (Cnt)	45.10	69.72^{b}	81.07	99.69	99	88	19.15^{b}	492.49^{a}
2 (Nacl)	45.72	71.50^{a}	81.60	99.68	94	85	19.09^{b}	491.37^{b}
3 (BA)	45.50	70.70^{ab}	81.21	99.76	99	92	19.32^{a}	491.62^{ab}
SEM	0.34	0.39	0.45	0.15	1.16	3.36	0.051	0.28
p-value	0.28	0.03	0.17	0.58	0.35	0.80	0.05	0.05

Cnt= control BA= Butyric acid

Table 3: Effects of butyric acid injection on body weight and FCR

	BW (g)				MBW (g)		FCR		
	----------				----------		----------		
Treat	one day	3 day	7 day	10 day	0-7 day	0-10 day	0-3 day	0-7 day	0-10 day
1 (Cnt)	59.27	106.6	215.5	297.5^{ab}	24.35	28.37	0.515	0.79^{ab}	0.95
2 (Nacl)	59.63	104.4	208.6	280.1^{b}	24.18	27.76	0.517	0.81^{a}	0.99
3 (BA)	59.63	108	228.4	304.6^{a}	23.89	27.55	0.482	0.73^{b}	0.91
SEM	0.83	1.36	6.36	7.4	0.27	0.37	0.01	0.02	0.02
p-value	0.94	0.23	0.11	0.05	0.51	0.34	0.29	0.05	0.14

BW= Body weight MBW= Mean body weight FCR= Feed Conversion Ratio

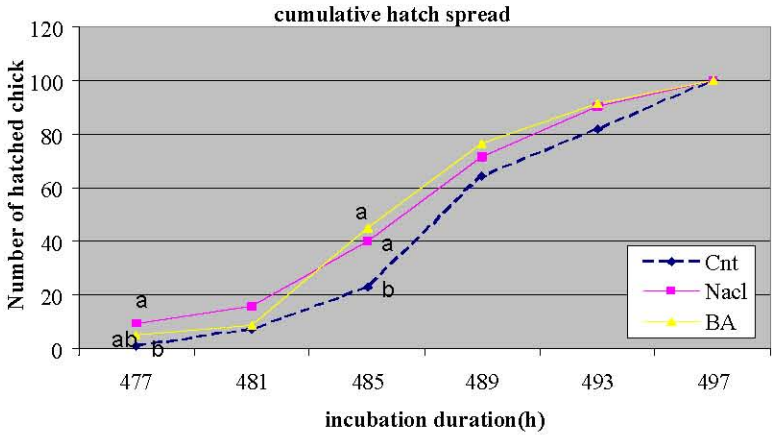

Chart 1: Effects of Butyric acid (BA) injection on hatch spread (Cumulative)

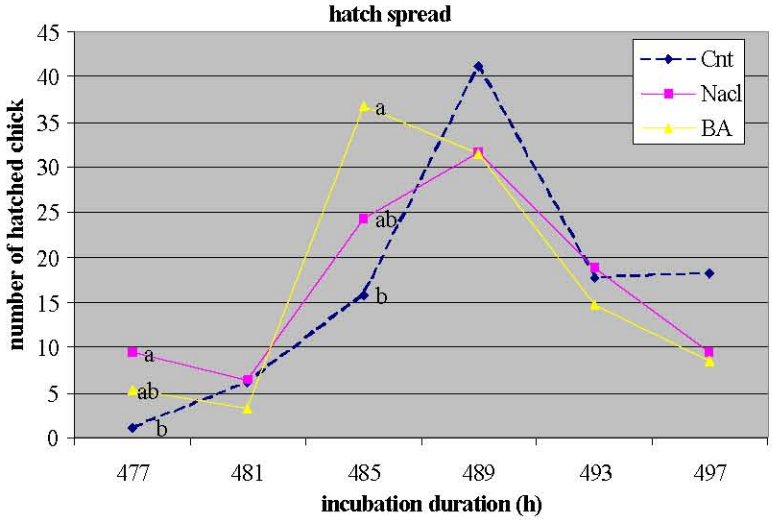

Chart 2: Effects of Butyric acid (BA) injection on hatch spread (non cumulative)

Table 4: Effects of Butyric acid (BA) injection on chick and intestine length

Treatment	Day	1 (Cnt)	2 (Nacl)	3 (BA)	SEM	p-value
chick length (cm)	0 (Hatch)	19.15^{b}	19.09^{b}	19.32^{a}	0.05	0.05
	3	22.82	22.35	22.3	0.21	0.19
	7	28.17	28.6	28.67	0.33	0.52
Duodenum length (cm)	0 (Hatch)	7.4	7.6	7.7	0.34	0.69
	3	14.15^{b}	15.87^{a}	15.62^{a}	0.32	0.006
	7	17.87	18.5	17.07	0.56	0.24
Jejunum length (cm)	0 (Hatch)	16.77^{ab}	15.72^{b}	20.52^{a}	1.31	0.05
	3	34.52	34.92	35.25	1.26	0.48
	7	41.97	44.37	41.25	1.78	0.59
Ileum length (cm)	0 (Hatch)	12.27	13.1	13.27	1.19	0.38
	3	33.5	31.25	31.97	1.18	0.28
	7	39.47	41.1	39.52	3.13	0.67
Intestine length (cm)	0 (Hatch)	36.45^{b}	36.42^{b}	41.50^{a}	0.88	0.003
	3	82.17	82.05	82.85	2.42	0.89
	7	99.32	103.97	97.85	0.38	0.17

Table 5: Effects of Butyric acid injection on carcass and organ weight

Treatment	Day	1 (Cnt)	2 (Nacl)	3 (BA)	SEM	p-value
YSW	0 (Hatch)	6.1	5.55	6.27	0.67	0.21
	3	0.58[a]	0.46[b]	0.54[ab]	0.03	0.05
	7	0.21	0.22	0.157	0.06	0.62
YFBM	0 (Hatch)	38.34[b]	40.79[a]	40.11[ab]	0.67	0.05
	3	110.59	105.27	107.21	1.75	0.14
	7	221.54	212.02	227.84	4.89	0.12
Breast wt	0 (Hatch)	3.21	3.61	3.18	0.187	0.82
	3 day	10.48	9.27	10.7	0.6	0.35
	7 day	41.12	37.67	41.15	0.84	0.22
Thigh wt	0 (Hatch)	6.25	6.63	6.46	0.18	0.79
	3	15.63	14.85	15.35	0.31	0.23
	7	35.10[ab]	33.16[b]	37.38[a]	0.75	0.05
Liver wt	0 (Hatch)	1.35	1.48	1.48	0.09	0.61
	3	6.4	6.57	6.58	0.45	0.18
	7	12.69[ab]	11.27[b]	14.18[a]	0.77	0.05
Heart wt	0 (Hatch)	0.35	0.33	0.36	0.03	0.26
	3	1.08	0.95	1.06	0.05	0.26
	7	2.34[ab]	2.08[b]	2.63[a]	0.16	0.05
Duodenum wt	0 (Hatch)	0.30[b]	0.33[ab]	0.40[a]	0.03	0.05
	3	2.19	2.28	2.92	0.3	0.65
	7	3.8	3.57	3.91	0.3	0.196
Jejunum wt	0 (Hatch)	0.43	0.47	0.53	0.05	0.396
	3	5.9	5.78	5	0.61	0.21
	7	7.4	7.66	7.2	0.61	0.43
Ileum wt	0 (Hatch)	0.33	0.31	0.27	0.04	0.74
	3	5.06	4.13	4.17	0.27	0.31
	7	5.7	6.19	6.36	0.45	0.57
Intestine wt	0 (Hatch)	1.06	1.12	1.21	0.07	0.7
	3	13.15	12.19	12.1	0.84	0.17
	7	16.91	17.43	17.48	0.89	0.06
Breast wt YFBM	0 (Hatch)	8.37	8.86	7.92	0.36	0.28
	3	9.52	8.8	9.98	0.57	0.11
	7	18.52	17.76	18.07	0.35	0.28
Thigh wt YFBM	0 (Hatch)	16.32	16.26	16.14	0.44	0.14
	3	14.14	14.11	14.33	0.28	0.1
	7	15.84	15.66	16.41	0.36	0.63
Liver wt YFBM	0 (Hatch)	3.52	3.63	3.72	0.22	0.32
	3	5.78	6.23	6.14	0.43	0.45
	7	5.73	5.31	6.24	0.07	0.77
Heart wt YFBM	0 (Hatch)	0.91	0.81	0.9	0.06	0.42
	3	0.98	0.9	0.99	0.04	0.06
	7	1.06	0.98	1.15	0.28	0.75

YSW=yolk sac weight YFBM=yolk free body mass Wt =weight

As shown in chart 1 and 2 there is significant effect of butyric acid treatment on spread of hatch between 477 and 485 hours of incubation. The highest number of chicks hatched at 485 hours from butyric acid injected eggs. Injection of butyric acid compared to control group increased small intestine length ($P<0.01$) and jejunum length ($p< 0.05$) of hatched chicks (Table 4). But duodenum length was not affected at hatch day. Results showed that significant effect of butyric acid injection on the yolk sac weight (YSW) at 3 day ($p< 0.05$) and in saline (Nacl) solution treatment was less than other groups, but injection effect on YSW in 0 and 7 days was not significant (Table 5). Injection of butyric acid on yolk free body mass (YFBM), duodenum weight on hatch (0) day, thigh weight, heart and liver weight at 7 day was significant ($p< 0.05$). Effect of injection of butyric acid on weight of other segments of small intestine in other days was not significant.

DISCUSSION

Hatching Parameters

Hatchability: Effect of *in ovo* feeding of butyric acid (HydroC4-30) on hatchability and saleable hatch percentage was not significant. Also the highest rate of saleable chicks was obtained in butyric acid treated group. These results are in agreement with other findings. Mousavi *et al.* [26] showed that injection of 1 ml Baby C4® in 18th of incubation had no significant effects on hatchability. Our result was in agreement with Nouboukpo *et al.* [27] which found no significant effect of *in ovo* injection of L-carnitine at 18 d of incubation on hatchability. Also in an experiment conducted by Ohta *et al.* [16] injection of amino acids solution into yolk sac at 7th d of incubation had no effect on hatchability. One of the important factors may affect embryo mortality is osmolality of solution, the maximum osmolarity of *in ovo* solution was 500-600 miliosmol suggeste by Uni and Ferket [14]. Osmolality of butyric acid solution in the current study was 0.028 mol/ L (280 mili osmol) which was far lower than Uni and Ferket [14] recommendation although the effects was not significant. Injection of 1 ml saline 0.9% solution reduced hatchability rate by 5%. This finding is in contrast with other reports that injection of 0.5 ml of Nacl 0.5% had no effect on hatchability [28, 29]. Glucose injection into amniotic fluid increased osmotic pressure and decreased hatchability [30]. The best hatchability was (more than 90%) with lower than 0.4 ml for fructose and sucrose and lower than 0.7 ml for dextrin, maltose and glucose. Then volume for injection is very important [31], because hatch fertile had negative relationship with injection volume [32].

Hatch Spread and Incubation Duration: In the current experiment incubation duration in butyric acid treatment was 0.87 h (52.2 minute) lower than control group. Also *in ovo* injection of butyric acid (HydroC4-30) solution induced lower incubation duration than control group (chart 1 and 2). Our finding was inconsistent with results from other researchers. Nouboukpo *et al.* [27] reported that L-Carnithine injection on 18th days of incubation delayed incubation time 4 hours in compare with control group. They attributed the prolonged incubation time to stimulating of fat metabolism, because it needs more time to affect the lipid metabolism by injection of L-Carnithine [27]. Also there was negative quadratic relationship between chick relative growth (RG) and incubation duration ($P< 0.05$) and incubation duration may influence postnatal juvenile growth [22]. It has been suggested that chick performance and growth after hatch affect adversely by feed intake [33].

Chick Weight and Quality: In the current study, butyric acid injection into fertile eggs had no significant effect on chick (body) weight at hatch and 3 and 7 days post-hatch, but body weight at 10 d of age was significant ($P < 0.05$). Meanwhile butyric acid treatment tended to increase chick weight 0.88, 1.31 and 2.38% compared to control group at hatch 3 and 10 days post hatch, respectively. Our finding was consistent with others. Baby C4® injection into amniotic fluid in 18th days increased body weight (by 3%) during 1-21 days in compare with control group [26]. In the experiment of Uni *et al.* [29]; Foye *et al.* [34] *in ovo* feeding carbohydrate were increased 5 to 6% of chicken and turkey body weight. Also dietary supplementation of Baby C4® improved 0.2% broiler body weight during 0 to 42 days [35] and dietary addition of butyric acid glycerides (2g/kg feed) had good results in compare with other levels and increased chick weight [6, 11]. But this level was not adequate to achieve the best performance [36]. Also dietary addition of butyric acid glycerides enhanced final body weight of by 11% compared with control [37]. Antongiovanni *et al.* [11] showed that only butyric acid level in the small intestine increased in early life of chicks due to dietary addition of Baby C4 and then decreased [11]. Mahdavi and Torki [8] reported that butyric acid application, could not obvious and positive effects on broilers that raise under clean environmental condition. Dietary sodium butyrate supplementation improved the growth performance in chickens under stress (disease) and this result attributed to the enhanced immune response and reduced tissue damage [38]. We speculate therefore that effects of *in ovo* injection of butyric acid in dirty eggs may be more pronounced compared to clean eggs.

Chick Length: Effect of *in ovo* feeding of butyric acid on chick length at hatch day was significant ($P < 0.05$). Also chick length 0.17cm (0.88%) at hatch (0) day in butyric acid injection treatment was more than control. This positive effect of injection on chick length could be justify butyric acid effects on body weight and chick quality. It is believed that increased in chick length (one of important factor in chick quality basis on hatchtech method), may lead to higher slaughter weight [39]. Also small chicks with longer body length, had better and more development of liver, heart and spleen [40].

Post-Hatch Performance: Effect of butyric acid injection on feed conversion ratio (FCR) at 0-7 days was significant ($p< 0.05$). Also FCR between 0-7 and 0-10 days was 0.06 and 0.04 in butyric treatment better than control. This study consistent with others result, similar to this experiment *in ovo* feeding of glutamine had no significant effect on FCR [30, 41]. We expected that with *in ovo* feeding, digestive tract development and growth increased and then FCR enhanced. It is important that FCR affected both body weight gain (BWG) and feed intake and in starter period. Volatile fatty acids improve feed conversion 0.03 and they described it is related to increase the permeability of bacterial cell wall and inactivity of the enzyme system and finally bacteria death [42].

Carcass Characteristics: Small intestine weight and length: Injection of butyric acid could effects on duodenum weight at hatch day significantly and it is 0.1 g more than control group. There were not any research about small intestine weight and length and effects of injection on both parameters. Glutamine injection into duck eggs increased intestine weight [43]. Also a mixture of 0.2% probiotic and 0.4% organic acid had not significant effects on large intestine, but small intestine was affected significantly [44]. Intestine development [28] and gastrointestinal tract capacity increased with *in ovo* injection [45]. Also increase resistance to infectious diseases in birds. Injection of butyric acid improved duodenum length (0.3 g, 4.05%), (1.47g, 10.38%), jejunum length (3.75 g, 22.36%), (0.73g, 2.11%) and small intestine (5.05g, 13.85%), (0.68g, 0.83%) at hatch (0) and 3 days post-hatch. But injection effects were significantly on duodenum, jejunum and small intestine length at 3, 0 (hatch) and 0 (hatch) days respectively. Thus increase in small intestine length caused to increased of absorption and chicken growth.

Breast and Thigh Weight: Injection of butyric acid could to increase thigh weight at 7 days post hatch significantly and thigh weight at 7 days was 2.28 g or 6.49% more than control. Also breast weight in 3 days post hatch was 0.22 g or 2.09% more than control but was not significant. *In ovo* feeding could to increased breast meat yield, Muscle development, health status and immune system of chicks [14, 29]. Glutamine, sucrose and maltose injected to amniotic of duck eggs on 23 days of incubation caused to increased breast weight 24 and 15% increased on 25 days of incubation and hatching time compare with control and this superior continue until 7 days of post hatch [43]. Other research showed that increased of percentage breast weight with protein injection in turkey eggs [34] and chicken eggs with injection of carbohydrate and beta-hydroxy butyrate increased breast weight on hatch, 10 and 25 days post hatch [29]. One of the most important aspect of *in ovo* feeding technology aspects increase in body weight on hatch day and increase breast weight and muscle development that finally decreased production cost of chicken meat. Also *in ovo* feeding injection and supply of nutrients caused to prevention of chicken starvation and gluconeogenosis [43]. Also injection to increased liver glycogen and muscle glycogen reserves compare with control [46].

Liver and Heart Weight: *In ovo* injection of butyric acid was improved liver weight to 0.13 (9.62%), 0.18 (2.81%) and 1.49 g (11.74%) in compare with control group at hatch, 3 and 7 days post hatch respectively. Also liver weight to yolk free body mass at 0, 3 and 7 days was 0.2, 0.36, 0.51 g or 5.68%, 6.22%, 8.90 % more than control group respectively. Treatment effects on heart weight was 0.01 (2.85%), 0.29 g (12.39%) at hatch and 7 days more than control. In other researches application of 0.2% probiotic and 0.4% organic acid in broiler ration could not effects on liver, gizzard and large intestine weight [44]. Also glutamine injection [41], L-Arginine and L-Carnithine [47] could not effect on visceral organ weight, carcass characteristics and organ weight to body weight ratio. But butyric acid injection on 16^{th} day of incubation caused to increase liver weight, crop and gizzard, small intestine weight and length [48]. Early injection of butyric acid maybe help to butyric acid consumption as a energy source and embryo had enough time to absorption of this sources, then organ development specially liver and heart and spleen had better development in longer chicks [40].

CONCLUSION

Therefore *in ovo* feeding of butyric acid could increase small intestine length and improve absorption nutrient and finally increase chick weight at 10 day post-hatch significantly. Also positive effects butyric acid on chick quality and chick length causes better growth chick at post-hatch. It was concluded from the results of current experiment that *in ovo* injection of butyric acid could improve hatching parameters and performance.

REFERENCES

1. Bolton, W. and W.A. Dewar, 1965. The digestibility of acetic, propionic and butyric acids by the fowl. British Poultry Science, 6: 103-105.
2. Hosseini Mansoub, N., K. Rahimpour, L. Majedi asl, M.A. Mohammad Nezhady, S.L. Zabihi and M. Mohammadi Kalhori, 2011. Effect of Different Level of Butyric Acid Glycerides on Performance and Serum Composition of Broiler Chickens. World Journal of Zoology, 6(2): 179-182.
3. Sakata, T., M. Adachi, M. Hashida, N. Sato and T. Kojima, 1995. Effect of n-butyric acid on epithelial cell proliferation of pig colonic mucosa in short-term culture. Deutsche Tierarztliche Wochenschrift, 102: 163-164.
4. Pryde, S.E., S.H. Duncan, G.L. Hold, C.S. Stewart and H.J. Flint, 2002. The microbiology of butyrate formation in the human colon. FEMS Microbiology Letter, 217: 133-139.
5. Galfi, P. and J. Bokori, 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. Acta Veterinaria Hungary, 38: 3-17.
6. Leeson, S., H. Namkung, M. Antongiovanni and E.H. Lee, 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poultry Science, 84: 1418-1422.
7. Van Immerseel, F., J.B. Russell, M.D. Flythe, I. Gantois, L. Timbermont, F. Pasmans, F. Haesebrouck and R. Ducatelle, 2006. The use of organic acids to combat Salmonella in poultry: A mechanistic explanation of the efficacy. Avian Pathology, 35: 182-188.
8. Mahdavi, R. and M. Torki, 2009. Study on usage period of dietary protected butyric acid on performance, carcass characteristics, serum metabolite levels and humoral immune response of broiler chickens. Journal of Animal and Veterinary Advance, 8(9): 1702-1709.
9. Antongiovanni, M., A. Buccioni, S. Minieri, I. Galigani and S. Rapaccini, 2010. Monobutyrine: a novel feed additive in the diet of broiler chickens. Italian Journal of Animal Science, 9(4): 1-8.
10. Friedman, A. and E. Bar-Shira, 2005. Effect of nutrition on development of immune competence in chickens gut associated lymphoid system. 15 th Eur. Symp. Poultry Nutr., Balatonfüred, Hungary, pp: 234-242.
11. Antongiovanni, M., A. Buccioni, F. Petacchi, S. Leeson, S. Minieri, A. Martini and R. Cecchi, 2007. Butyric acid glycerides in the diet of broiler chickens: effects on gut histology and carcass composition. Italian journal of Animal Science, 6: 19-25.
12. Van der Wielen, P.W.J.J., S. Biesterveld, S. Notermans, H. Hofstra, B.A. Urlings and F. VanKapen, 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Applied Environment Microbiology, 66: 2536-2540.
13. SILOSPA, 2011. Monobutyrin-Hydro C4. Firenze. Italy. http://www.silosrl.com/?action=zoo and sezione=prodotti and idprodotto=10.
14. Uni, Z. and P.R. Ferket, 2003. Enhancement of development of oviparous species by *in ovo* feeding. US Regular Patent US 6,592,878. North Carolina State Univ., Raleigh and Yissum Res. Dev. Co. of the Hebrew Univ. Jerusalem, Jerusalem, Israel.
15. Zhai, W., S. Neuman, M.A. Latour and P.Y. Hester, 2008. The effect of *in ovo* injection of LCarnitine on hatchability of white leghorns. Poultry Science, 87: 569-572.
16. Ohta, Y., N. Tsushima, K. Koide, M.T. Kidd and T. Ishibashi, 1999. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poultry Science, 78: 1493-1498.
17. Careghi, C., K. Tona, O. Onagbesan, J. Buyse, E. Decuypere and V. Bruggeman, 2005. The effects of the spread of hatch and interaction with delayed feed access after hatch on broiler performance until seven days of age. Poultry Science, 84: 1314-1320.
18. Tullet, S., 2009. Investigating hatchery practice. Ross Technology, Scotland, Uk. October, pp: 1-44.
19. Kawalilak, L.T., A.M. Ulmer Franco and G.M. Fasenko, 2010. Impaired intestinal villi growth in broiler chicks with unhealed navels. Poultry Science, 89: 82-87.
20. Willemsen, H., N. Everaert, A. Witters, L. De Smit, M. Debonne, F. Verschuere, P. Garain, D. Berckmans, E. Decuypere and V. Bruggeman, 2008. Critical assessment of chick quality measurements as an indicator of posthatch performance. Poultry Science, 87: 2358-2366.

21. PasReform, 2008. Incubation Guide Broiler. PasReform, Zeddam, The Netherlands.
22. Tona, K., F. Bamelis, B. De Ketelaere, V. Bruggeman, V.M.B. Moraes, J. Buyse, O. Onagbesan and E. Decuypere, 2003. Effects of egg storage time on spread of hatch, chick quality and chick juvenile growth. Poultry Science, 82: 736-741.
23. Miller, Carin R., 2007. Developmental Gene Expression of Nutrient Transporters in the Small Intestine of Chickens from Lines Divergently Selected for High or Low Juvenile Body Weight. Msc thesis. Virginia Polytechnic Institute and State University.
24. Aviagen, 2009. Ross broiler management manual, pp: 1-114. Scotland, UK. www.aviagen.co
25. SAS Institute, 2004. SAS/STAT User's Guide, Release 9.1 ed. SAS Institute Inc., Cary, NC.
26. Mousavi, S.N., M. Shivazad, M. Chamani, A.A. Sadeghi and H. Lotfollaheian, 2010. Effects of *In ovo* Injection of Amino Acids, Carbohydrates, butyric acid in broiler breeder eggs on small intestine morphology and performance of chicken. 4th animal science congress. IRAN (Persian).
27. Nouboukpo, K.E., K. Tona, B. Kamers, N. Everaert, H. Willemsen, O.M. Onagbesan, M. Gbeassor and E. Decuypere, 2010. Effects of *in ovo* Administration of L-carnitine on Hatching Events and Juvenile Performance of Layer-type Chick. International Journal of Poultry Science, 9(10): 980-983.
28. Tako, E., P.R. Ferket and Z. Uni, 2004. Effects of *In ovo* feeding of carbohydrates and β-Hydroxy-β-Methylbutyrate on the development of chicken intestine. Poultry Science, 83: 2023-2028.
29. Uni, Z., P.R. Ferket, E. Tako and O. Kedar, 2005. *In ovo* feeding improves energy status of lateterm chicken embryos. Poultry Science, 84: 764-770.
30. Pedroso, A.A., L.S. Chaves, M.B. Cafe, N.S.M. Leandro, J.H. Stringhini and J.F.M. Menten, 2006. Glutamine as broilers embryos nutrient. Page 43 in APINCO Conference on Avian Science and Technology, Santos. Fundação Apinco de Ciéncia e Tecnologia Avicolas, Campinas, São Paulo, Brazil.
31. Ferket, P.R., O. Foye, J. De Oliveira, E. Tako and Z. Uni, 2005. *In ovo* nutrition: Impact on gene expression, gut development and growth performance. Arkansas Annual Animal Nutrition Conference.
32. Zhai, W., D.E. Rowe and E.D. Peebles, 2011. Effects of commercial *in ovo* injection of carbohydrates on broiler embryogenesis. Poultry Science, 90: 1295-1301.
33. Pinchasov, Y. and Y. Noy, 1993. Comparison of post-hatch holding time and subsequent early performance of broiler chicks and turkey poults. British Poultry Science, 34: 111-120.
34. Foy, O.T., Z. Uni and P.R. Fekret, 2006. Effect of *in ovo* feeding egg white protein, β-Hydroxy-β-Methyl butyrate and carbohydrates on glycogen status and neonatal growth of turkeys. Poultry Science, 85: 1185-1192.
35. Hosseini Mansoub, N., 2011. Comparitive Effect of Butyric Acid and Probiotic on Performance and Serum Composition of Broiler Chickens. Advances in Environmental Biology, 5(6): 1188-1191.
36. Panda, A.K., S.V. Rama Rao, M.V.L.N. Raju and G. Shyam Sunder, 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens Asian-Australian journal of Animal Science, 1: 1-9.
37. Roth, N., 2009. Better Gut Health Enhances Animal Performance. www. The poultrysite.com.
38. Zhang, W.H., Y. Jiang, Q.F. Zhu, F. Gao, S.F. Dai, J. Chen and G.H. Zhou, 2011. Sodium butyrate maintains growth performance by regulating the immune response in broiler chickens. British poultry Science, 52(3): 292-301.
39. Molenaar, R. and I. Reijrink, 2009. Chick length and Organ development. Newsletter, HatchTech Incubation Technolgy. Netherlands. www.hatchtech. nl
40. Hill, D., 2001. Chick length uniformity profiles as afield measurement of chick quality? Avian Poultry Biology Review, 12: 188.
41. Dos Santos, T.T., A. Corzo, M.T. Kidd, C.D. McDaniel, R.A. Torres Fillho and L.F. Araujo, 2010. Influence of *in ovo* inoculation with various nutrients and egg size on broiler performance. The Applied Poultry Research Journal, 19: 1-12.
42. Hesseling, H.K., D.J. Langhout and P. Wijitten, 2002. Volatile fatty acids and essential oils (BIACID) improve technical performance of broiler. Provimi Research and Technology Center. Brussels, Belgium.

43. Chen, W., R. Wang, H.F. Wan, X.L. Xiong, P. Peng and J. Peng, 2009. Influence of *in ovo* injection of glutamine and carbohydrate on digestive organs and pectoralis muscle mass in the duck. British poultry Science, 50(4): 436-442.
44. Çelik, K., M. Mutluay and A. Uzatici, 2007. Effects of probiotic and organic acid on performance and organ weights in broiler chicks. Archiva Zootechnica, 10: 51-56.
45. Smirnov, A., E. Tako, P.R. Ferket and Z. Uni, 2006. Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by *in ovo* feeding of carbohydrates. Poultry Science, 85: 669-683.
46. Foye, O., P. Ferket and Z. Uni, 2003. The effects of *in ovo* feeding of protein and beta-methyl-betahydroxybutyrate (HMB) on early growth and glycogen status of turkey poults. Poultry Science, 82: 11.
47. Kunjing, S.H.I., W.U. Lingying and T.A.N. Rongbing, 2008. Effects of *in ovo* Feeding L-arginine and L-ornithine on the Early Development, Immune Organ and Hormone of Plasma of Broiler. China Poultry, 17.
48. Gonzales, E., A.S. Oliveira, C.P. Cruz, N.S.M. Leandro, J.H. Stringhini and A.B. Brito, 2003. *In ovo* administration of butyric to broiler embryos. Rua Jastino Miranda Camarago, 1386: 16610-130.