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EFFECT OF *IN OVO* SUPPLEMENTATION OF HONEY IN FERTILE EGGS ON POST HATCH GROWTH PERFORMANCE OF BROILER CHICKENS

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ABSTRACT

The present study was designed to evaluate the effects of *in ovo supplementation* of honey in fertile eggs on the post hatch growth performance of broiler chickens. In this regard, 120 fertile eggs were divided into 3 treatments and a control group. *In ovo* (20%) supplementation of honey at 0.5ml/egg was inoculated into yolk sac in each treatment group on day 15, 16 and 17 of incubation and the observations were made immediately after hatch, at the end of the brooding period and at the time of harvesting. The birds were observed for hatchability percentage and growth performance parameters. Our results showed no significant difference in the hatchability percentage (84 for non-injected control, 84, 90, 94% for honey injected on day 15,16 and 17 respectively) and hatchling weight (43.9,44.5,43.8 and 44.56g) among different groups. Similarly, heart weight (0.372, 0.362, 0.364 and 0.416g), intestinal weight (2, 2, 1.89 and 1.76g) and yolk weight (4.2,4.5, and 5.0g) were also non-significant among the groups. However, liver weight (1.31,1.34,1.06 and 1.13g; P< 0.0002) was significantly different among the different groups in day old chick. Day old chicken intestinal villi length (88.7, 83, 93 and 104 µm; P<0.0000) showed marked difference among the groups. Moreover, treated groups at the end of the 14th day, also demonstrated no significant difference in the body weight, carcass weight and liver weight, however, intestinal villi length (392, 414, 429 and 469 (µm); P<0.0000) was significantly different among the groups. But at marketing stage, significant difference was observed among treated and control groups, in the average weight gain (1761g, 1735g, 1918g, and 1939g; P<0.0014), carcass weight (928, 890, 1010, and 1023g; P<0.0033) and gizzard weight (49.9, 46.5, 54.6, and 55g; P< 0.0007). The feed efficiency values were significantly better in the honey inoculated groups than the non-treated group. It is concluded that *in ovo* supplementation of honey in fertile eggs can increase the size of visceral organs and improve post hatch growth performance of broiler chicks.

Keys word: *In ovo*, honey, broiler, embryo, growth.

INTRODUCTION

In ovo feeding is the administration of exogenous nutrients into avian fertile eggs (Uni *et al.*, 2005). *In ovo* supplementation involves the administration of a solution or suspension of nutrients together with other natural compounds to modulate enteric development, to improve the hatchling's nutritional status during the transition from embryonic nutrition and to transfer chicken into the diet digestive competency. Naturally the developmental environment of chicken embryo has limited amount of *in ovo* energy and nutrients to support embryonic growth and hatching percentage (Foye *et al*., 2006). Moreover, before resuming to exogenous feeding, chick growth depends on the nutritional supplements absorbed from the residual yolk sac. The previous reports have indicated that *in ovo* feeding may serve as a tool to overcome early growth constraints during embryonic and post-hatch development in poultry and supplying embryos with exogenous nutrients *in ovo* may also improve hatchability and increase hatched chick weight and post-hatch development (Uni *et al.*, 2005). Moreover, *in ovo* delivery of nutrients along with enteric modulators into the amniotic fluid results in the improved early development of the digestive organs during the last quarter of embryonic development.

The first *in ovo* feeding was reported by Uni and Ferket in 2003 by administrating high volume (0.4 -1.2 ml) of *in ovo* supplements to the amniotic fluid of chicken and turkey eggs to observe embryonic growth. Later, *in ovo* feeding was considered as a future commercial approach to develop embryonic weight, intestinal health, bone, pectorals muscle and for FCR improvement (Tako *et al*., 2004; Smirnov, *et al*., 2006; Bohorquez, *et al*., 2007, Moore *et al.,* 2005; Kornasio *et al.,* 2011).

A variety of nutrient supplements can be included in the *in ovo* supplementing solution in various arrangements and compositions after thorough study of their transportation and assimilation mechanism such as Na^+ and Cl^- ions play main role in the activity of apical and transporters, and in the amino acids, and glucose absorption. Additionally, glucose storage as glycogen is a very important energy source for maintaining normal

metabolism and body growth during pre- and post-hatching days (Christensen *et al.,* 2000). Besides, broiler embryos were fed *in ovo* with 1 ml saline solutions containing maltose, sucrose, and dextrin at 17th days of incubation with improved growth rate (Tako *et al.*, 2004).

So far carbohydrates, sucrose, maltose, NaCl, arginine, dextrin beta-hydroxy-beta-methyl butyrate, and Zn-methionine egg white protein has been reported as *in ovo* feed supplement (Kadam *et al*., 2013, Joshua *et al.,*2016, L. Zhang *et al*.,2016, Goel, *et al*. 2016, Khaligh *et al*.,2018, Sun *et al*. 2018). However, there are abundant natural sources present in the nature with the unidentified growth factors which are yet to be explored. Thus, we have selected honey for *in ovo* supplementation due to its high nutritional profile i.e., mainly of essential nutrients containing only trace amounts of protein, dietary fiber and vitamins; Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Pyridoxine (B6), Folic acid (B9) and Vitamin C (León-Ruiz, *et al*. 2013). Minerals; Zinc, Sodium, Potassium, Phosphorus, Magnesium, Calcium, Iron (Oroian, M *et al*.2015) and carbohydrates. Honey is a complete nutritional food made from flower's nectar collected by honey bees (*Apis mellifera*). Moreover, it contains fructose: 38.2%, Glucose: 31.3%, Maltose: 7.1% Sucrose: 1.3% Water: 17.2%, higher sugars: 1.5% and Ash: 0.2% (Ball, D.W.,2007). Additionally, honey feeding has shown the safety conformation of healthy individuals as it does not change the micro biota homeostasis in the gut.

Thus, the present study is aimed to observe *in ovo* effect of honey inoculation on the development of internal organs of broiler chickens and to observe *in ovo* effect of honey on post hatch growth performance of broiler chickens**.**

MATERIAL AND METHODS

One hundred and twenty fertile eggs (Hubbard breeder of age 34th weeks) were used and investigated in three stages i.e. Stage (i): Incubation period, Stage (ii) Brooding period, Stage (iii) Finisher period. Eggs were randomly divided into 4 groups with 3 replicates, (10 eggs/ replicate) were used for *in ovo* supplementation (20%) of honey at 0.5ml/egg inoculated into yolk sac at different incubation days i.e. group B (15th), C (16th) and D (17th) days of inoculation and group A (control). The birds were studied for *in ovo* supplementation of honey effect on hatchability percentage, hatching weight, carcass weight, blood glucose level and internal organ weight (heart, gizzard and intestine) during three stages of birds. Final body weight gain, feed intake, FCR and mortality percentage (%) were studied.

Incubation management: The Incubator (KHUL USA, Model AZYS 800-110) was used after thorough disinfection by water, ethanol and fumigation. Temperature of incubator was set at 99.5°F from day one to 18th day and then the temperature was adjusted at 98.5°F from 19th days till hatching. Humidity of incubator was set at 65% throughout the experiment. After through disinfection of fertile eggs, the complete fertile eggs were randomly divided into 4 groups with 3 replicates, (10 eggs/ replicate) and set in vertical position (pointed end down) into the incubator trays. For confirmation of the fertility 1st candling was performed at day 4th of incubation and at the time of *in ovo* supplementation then after 24 hours of honey inoculation in each group.

Procedure of *in ovo* supplementation: Initially, egg candling was performed to detect the site of *in ovo* supplementation site and then eggs were held in the specially designed egg holder with egg Candler. On the conformation of the embryonic position, eggs were disinfected with 70% ethanol swab and then punctured with sterilized egg shell boring needle gently. After the pore had been made, 0.5ml of 20% honey supplementation was inoculated with the help of disposable 24G needle into the egg yolk sac and then pore was sealed with melted liquid paraffin. The whole procedure was completed in 30 minutes.

Hatchling percentage (%): Hatchling percentage (%) was measured by the using following formula:

$$\text{Hatchling percentage (\%)} = \frac{\text{Total chicks hatched}}{\text{Total eggs set in incubator}} \times 100$$

Measurement of body weight, internal organs and yolk weight: weight of the organs was measured with ADAM Electric weighing balance Model AAA-250

Blood glucose level: The blood samples were collected directly from the heart of chicks and were analyzed for blood glucose level by GOD-PAP Method (1971), through Enzymatic Colorimetric Test for Glucose.

Length of intestinal villi: Histological analysis were performed for measuring intestinal villi length. For this purpose, intestine was washed with the cold PBS at 4 °C and then fixed in the cold 10% formaldehyde solution for 48 hours. After overnight soaking of the samples in the tap water for the removal of the formalin, samples were dehydrated in the graded solutions of the alcohol and xylene ford tissue embedding in the paraffin. The embedded tissues were sliced at 4 µm thickness and mounted on the glass slides. Paraffin wax was removed by melting and treating it in the xylene and gradient solution of ethanol and later staining with Harris Hematoxylin and eosin. The fixed slides were observed for the length of intestinal villi under microscope and pictures were captured with a digital camera and analyzed accordingly

Feed efficiency: Feed efficiency was calculated based on total feed intake by a chick for gaining 1000 grams weight:

$$FCR = \frac{\text{Total feed intake}}{\text{Total live body weight}}$$

Mortality percentage: Mortality percentage was calculated by using the following formula:

$$\text{Morality (\%)} = \frac{\text{Total number of dead broilers}}{\text{Total number of reared broilers}}$$

RESULTS

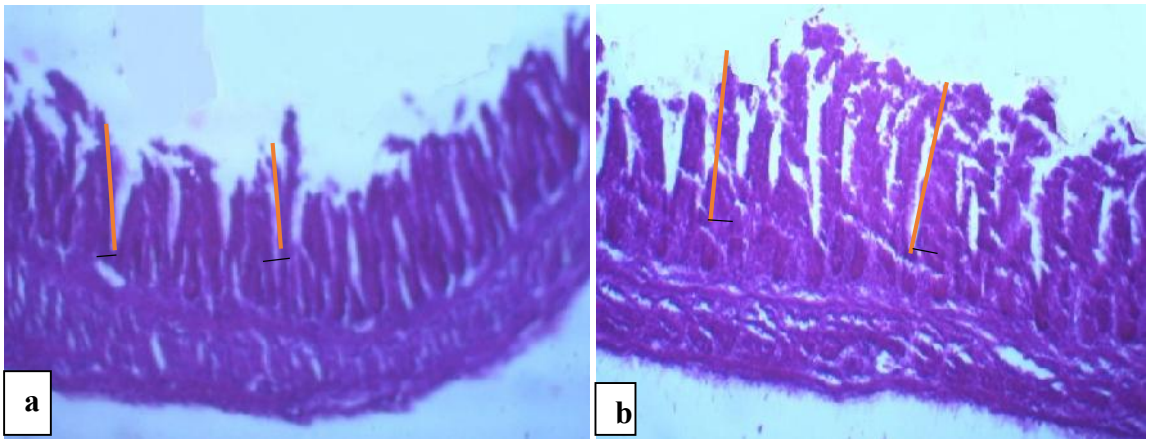
Effect of honey inoculation on DOC: Present study was conducted to define the effects of *in ovo* supplementation of honey in fertile eggs and on post hatch growth performance of broiler chickens. In this regard, our results for determining the effect of honey on egg inoculation on DOC showed no any significant difference on the hatchability % (table 1). There was also no any significant difference in the weight gain of DOC weight, heart weight, gizzard weight and intestinal weight. Results also revealed non-significant difference in the residual yolk weights among the treated and the control group. In addition to this, our results for blood glucose level (mg/dl) also showed non-significant difference among the treated and the control group. However, intestinal villi height and liver weight showed significant difference in the treated and control group. The weight of liver was significantly higher in the control and B groups than the C and D groups. Contrarily, group D intestinal villi height (104.30±2.6[a]) showed significant difference in comparison to other groups ($P<0.05$).

Effect of honey inoculation on the post hatch brooding period: Our results for determining the effect of honey inoculation at end of brooding stage showed no any significant difference for the live weight, carcass weight, live weight, heart weight, gizzard weight, intestine weight, despite numerical difference among the groups. Similarly, length of intestine showed no significant difference among the treated and control group. However, intestinal villi height at this stage showed significant difference in the D groups (68.99±3.5[a]) in comparison to the C, B, and A group.

Table.1 Effect of honey inoculation on DOC

Parameters	Groups			
	A	B	C	D
Hatchability %	84	84	90	97
Weight of DOC (g)	43.99±1.8	44.53±2.2	43.83±2.48	44.56±1.5
Liver weight (g)	**1.31±0.05[a]**	**1.342±0.10[a]**	1.064±0.02[b]	1.132±0.12[b]
Heart weight (g)	0.372±0.03	0.362±0.04	0.364±0.03	0.416±0.02
Gizzard weight (g)	1.85±0.16	1.984±0.10	1.982±0.09	2.044±0.08
Intestinal weight (g)	2±0.2	2.01±0.39	1.89±0.2	1.76±0.15
Residue yolk weight (g)	4.22±1.70	4.47±1.75	5.06±0.7	5.01±0.15
Height of villi (µm)	88.67±2.6[b]	82.98±2.08[c]	92.70±2.88[b]	**104.30±2.6[a]**
Blood glucose level (mg/dl)	254.03±22.2	236.86±24.8	224.06±51.64	231.33±67.71

Values are Mean ± SE, Significance level $P < 0.05$



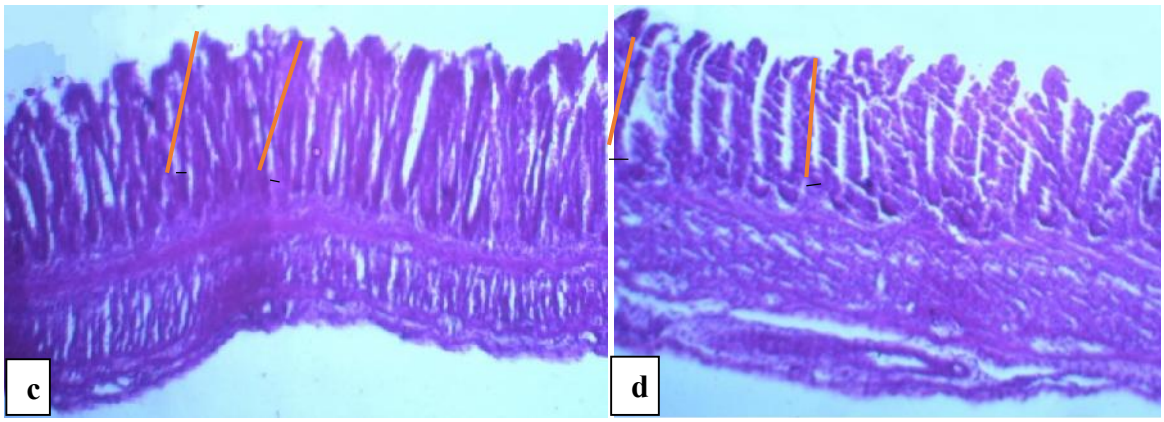


Figure 1. Group. A, B, C, D: Representing intestinal villus height in µm (day 1).

Table 2. Effect of honey inoculation on the post hatch brooding period.

Parameters	Groups			
	A	B	C	D
Liver weight (g)	218.582±12.93	227.943±14.4	246.86±24.35	243.442±31.73
Carcass weight (g)	143.7±8.02	147.99±13.45	160.32±11.95	152.71±15.55
Liver weight (g)	9.01±0.95	8.82±0.85	9.10±0.64	8.97±0.86
Heart weight (g)	2.775±0.15	2.78±0.17	2.803±0.15	2.818±0.2
Gizzard weight (g)	8.11±0.72	8.45±0.95	8.21±1	8.43±0.95
Intestine weight (g)	22.58±2.53	22.87±1.58	23.33±0.90	23.06±1.25
Length of intestine (cm)	126±3.28	126.16±4.8	129.16±1.84	129.33±1.5
Intestinal villi height (µm)	392.52±4.5^{d}	413.75±6.42^{c}	429.25±4.78^{b}	468.99±3.5^{a}

Values are Mean ± SE, Significance level P < 0.05

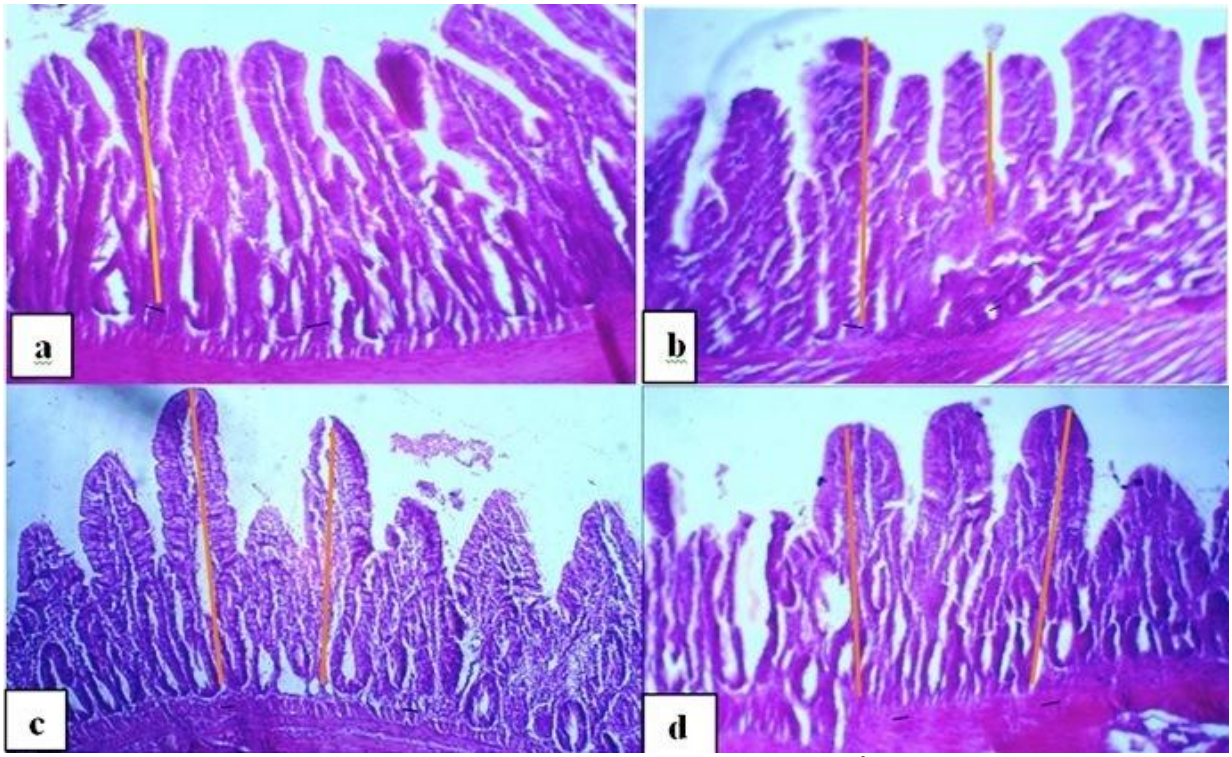


Figure: 2 Groups. A, B, C, D: Representing intestine villi height in µm (day 14th).

Figure: 2 Groups. A, B, C, D: Representing intestine villus height in µm (day 14th).

Effect of honey inoculation up to harvesting: The results for the *in ovo* honey inoculation up to the harvesting (42 days) indicated highly significant difference in the live body weight gain in the group D (1938.66±36.950[a]) in comparison to the Group A, B and C. Similarly, group D also showed highly significant difference for carcass weight, gizzard weigh in group D in comparison to other groups. However, there was no any statistically significant difference among the treated and control group for the feed intake, liver weight, heart weight, and intestinal weight. There was also significant difference in the feed conversion ration among the groups. Groups D showed better FCR (1.97±0.026[c]) then other groups. Moreover, length of intestine and mortality (%) also showed no any significant difference among the treated groups.

Table 3. Effect of honey inoculation up to harvesting.

Parameters	Groups			
	A	B	C	D
Live body weight (g)	1761.33±40.068[b]	1735±56.348[b]	1918.33±54.848[a]	**1938.66±36.950[a]**
Feed intake (g)	3851.33±42.224	3733.33±125.82	3925.66±82.585	3827.66±25.775
FCR	**2.18±0.029[a]**	2.15±0.042[ab]	2.04±0.061[bc]	1.97±0.026[c]
Carcass weight (g)	928±41.79[bc]	890±24.02[c]	1010.66±30.90[ab]	**1023.33±35.85[a]**
Liver weight (g)	49.87±1.40	47.32±1.38	52.77±2.89	53.90±3.88
Heart weight (g)	12.04±0.65	12.08±0.60	14.89±2.62	14.63±1.55
Gizzard weight (g)	49.88±1.41[b]	46.56±1.38[b]	54.64±2.89[a]	**54.99±3.88[a]**
Intestinal weight (g)	113.88±1.894	113.66±2.120	124.35±4.238	125.52±2.744
Length of intestine (cm)	156.48±7.70	164.83±1.17	176.63±4.99	181.87±4.06
Mortality %	6	5	1	1

Values are Mean ± SE, Significance level $P < 0.05$.

DISCUSSION

In the present study, we have evaluated the effects of *in ovo* supplementation of honey in fertile eggs on post hatch growth performance of broiler chickens at day old chicken stage, at the end of brooding period and at harvesting stage. For this purpose, we have investigated the birds for the hatchability percentage, hatching weight, carcass weights, internal organ weight (heart, gizzard and intestine), and final body weight gain, intestinal length, and villi size, blood glucose level, feed intake, FCR, and mortality percentage (%) at three stages of growth.

Hatchability indicates the percentage of eggs that are set to hatch chicks and can walk and eat. It is influenced by the number of factors, such as nutrient availability inside the egg, egg turning, and position of egg (Cardeal *et al*. 2015). Although statistically we have reported non-significant difference in the hatchability, however figuratively it was better than other groups., This has also been reported by Dong *et al.* 2013; Uni *et al*. 2005 and Khaligh *et al*., 2018. In the present study, slight increase in the hatchability percentage might be due to honey nutrients containing carbohydrates, amino acids, vitamins and minerals required for nutrient deficient embryo. However, due to no research regarding honey *in ovo* supplementation, it is difficult to understand that through what physiological mechanism honey improves hatchability percentage. Speculatively, it might be due to the embryo formed by the hybrid cross of two heavy chicken breeds which results in the deficiency of nutrients at the last stage of embryonic development. Thus, *in ovo* supplementation at the last stage results in the improved embryonic growth and hatchability with better organ formation. Thus, further research is required to fully understand the physiological mechanism that leads to the better hatchability. Moreover, our study showed no marked difference in the hatchling weight among the groups, contrarily to Foye *et al*. (2006) who used amino acid mixture and β-hydroxy-β-methyl butyrate, and carbohydrate on 17th day of incubation and reported significant increase in body weight through *in ovo* injection respectively. In our opinion, the growth of DOC might depend on the amount and the continuity of the dose as the smaller amounts and single *in ovo* dose might by consumed by the embryo early and leaving embryo again nutrient hungry. Thus, the inoculated dose might need increase in the dose and continuity. For this purpose, additional research is required to optimize the dose and timing.

Our results for the RYW showed minute difference among the groups, which is in accordance with the results McGruder *et al*. (2011), Gao *et al* (2018) and Zhai *et al*. (2011) who reported that *in ovo* injection have no any determinable effects on yolk weight. Thus, this slight difference might be due to the enrichment of embryonic energy from outside source and its usage as priority and reserving its own energy sources in the form of RYW. Additionally, our results showed no difference in the internal organ weight except in liver and gizzard, but this increased weight of liver was not maintained during brooding and finisher stages among the group. In

addition to this, no remarkable difference in the blood glucose levels were detected at DOC stage which is not in conformity with Bhanja *et al.* (2008) who reported marked rise in the blood glucose level in new hatched birds after *in ovo* feeding. Besides, our results for carcass weight were non-significant at DOC and at the end of brooding stage. However, carcass weight showed marked difference among the treatment and control groups. The similar findings have been reported by Kornasio *et al.* (2011) and Zahi *et al*. (2011) who reported that *in ovo* feeding had a positive effect on the weights of the *pectoris muscle* when supplemented by the CHO by *in ovo* supplementations. This increased weight of carcass may be due to the protection of glycogen reservoirs due to the extra supplementation of CHO in the *pectoris muscle* which is needed in the multiplication of myofibrils in this area.

Intestinal morphometry which includes intestinal length and digestive capacity showed significant difference at harvesting stage in the treatment groups when compared with control, which is supported by (Tako, *et al.*, 2004, Smirnov, *et al*., 2006; Bohorquez, *et al*., 2007). Increased length of intestine might be due to honey's antiseptic nature that might decrease the number of microorganism and increase cell proliferation that enhances the length of intestine. Similarly, body weight gain was higher in treated groups which is like the findings of previous observations (Tako *et al*. 2004; Bohorquez *et al.* 2007) and increased growth rate and feed efficiency (Kornasio *et al.* 2011, Leitao *et al.* 2010). This might be due to the improved intestinal health at brooding stage and thus increased digestion, and metabolism, and decrease chances of nutrients elimination through feces. *In ovo* honey supplementation also resulted in the better FCR in the treated groups**.** These results were supported by previous observations which stated that, *in ovo* injection improved feed efficiency (Uni and Frekat. 2003; Kornasio *et al.* 2011). Finally, lower mortality (%) was reported in treated groups which is in line with Uni and Ferket. (2003) who stated that mortality % was reduced through *in ovo* injection of amino acid and CHO. This might be due to the unidentified factors present in honey that improve the health parameters during embryogenesis.

Taken together, it is reported that *in ovo* supplementation of honey on day 17th of incubation improves the hatchability %, improves enteric health status, and improves feed efficiency and growth performance of broiler.

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