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Effect of *in ovo* Honey Administration on Fertility and Post Hatch Performance of Broiler Chickens

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Abstract | Supplementation of nutrients to growing embryo is known to cause various positive effects as the growing chicken embryo have inadequate quantity of nutrients and energy that may delay the growth and cause productive losses during post hatch life. The present study aimed to investigate the effect of *in ovo* feeding of honey on hatchability and post hatch performance of broiler chickens. Chicken embryos were inoculated with 0.5 mL honey (20% diluted) while control group was inoculated with same quantity of normal saline on day-15 of incubation. Upon hatching, day-old chicks of both honey treated and control group were transferred to experimental farm to determine their post hatch growth performance. The results showed that *in ovo* honey treatment significantly ($P < 0.05$) improved the hatchability percentage as well as birth weight of chicks as compared to saline treated group (control). Furthermore, honey administration improved ($P < 0.05$) the feed intake, body weight gain and carcass weight, while it improved ($P < 0.05$) FCR and reduced mortality in broiler chickens as compared to control group. These results demonstrated that *in ovo* supplementation of honey had better impact on hatchability and post hatch performance of broiler chickens.

Keywords | *In ovo*, Post hatch, Honey, Hatchability, Performance

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INTRODUCTION

During development of the neonatal period, anything that delays or stimulates growth will have an obvious effect on overall performance and health status of poultry (Ferket, 2006). It has been proposed that 21 days incubation period and early post hatch period of the chick has significant contribution (about 50%) in performance of broilers in commercial production system (Karadas et al., 2011). *In ovo* administration is a type of feeding of exogenous nutrients directly given to the chicken embryo in the form of a suspension or solution. This supplemental nutrition alters the enteric development and improve the hatchling's status, during the transition from embryonic nutrition to diet digestive competence (Foye et al., 2006; Uni and Ferket, 2002). Because the developmental environment of chicken embryo have insufficient nutrients and energy that may affect the production parameters during post hatch life (Gonzales et al., 2003).

During development, proper nutrition may help in regulating the immunity that cause the reduced incidence of diseases and increased the profitability of farmer (Ali et al., 2016; Korver and Klasing, 2001). Some natural products

such as prebiotics (Gibson et al., 1995), probiotics (Nichols and Andrew, 2007), herbal extracts (Chang et al., 2007) and enzymes (Eisenthal and Danson, 2002) have been reported in various model animals for immune enhancing effects. *In ovo* feeding has been reported to improve intestinal health (Tako et al., 2004; Smirnov, et al., 2006), bone mineralization (Luqman et al., 2021), embryonic weight, pectorals muscles and post hatch performance (Kornasio et al., 2011). In a recent review, *in ovo* is declared as a most recent and convenient approach to accomplish the nutritional requirements of hatchling embryo (El-Sabrout et al., 2019).

Honey is a viscous and sweet natural product that is formed from the nectar of flowers by honeybees (Alvarez-Suarez et al., 2014). It is an important insect-derived natural product that has been reported as a traditional therapeutic food since ancient time. It contains significant amount of proteins, vitamins, dietary fibers, minerals and various biologically active ingredients like flavonoids, polyphenols, aromatic compounds and diterpene acids (Lofty et al., 2006; USDA nutrients database, 2014). It has shown many biological activities, like antioxidation, anti-inflammatory, immunomodulatory and antimicrobial effects (Alvarez-Suarez et al., 2014; Saeed et al., 2017).

A number of nutrients like carbohydrates (Chen et al., 2009), ascorbic acid (Ipek et al., 2004), dextrin (Chen et al., 2009), glucose (Salmanzadeh, 2012), glycogen (Kornasio et al., 2011), glutamine (Santos et al., 2010), minerals (Yair et al., 2013) and multivitamin (Leitao et al., 2010) have been reported for their *in ovo* effects. However, little information is available on the *in ovo* effects of honey in chicken embryo. Keeping in view many health effects of honey we hypothesized that *in ovo* feeding of honey will improve the hatchling as well as performance of broilers. Therefore, the present study was designed to explore the effects of *in ovo* feeding of honey on hatchability, birth weight and post hatch performance of broiler chickens.

MATERIAL AND METHODS

Experimental design and *in ovo* feeding

The whole experimental protocol was approved by the Board of Advanced Studies, Sindh Agriculture University, Tandojam. A total of 160 fertile Hubbard breeder eggs (wt. 46-52 g) of 34 weeks age were purchased from a commercial breeder farm, and brought to the Poultry Experimental Station, SAU, Tandojam. The eggs were first fumigated, weighed, candled, and equally divided into two groups. The first group was inoculated with a 0.5 ml of diluted honey (20%; Marhaba Laboratories Lahore, Pakistan) while second group served as control and was inoculated with the same quantity of normal saline through injection in the yolk sac. The eggs were inoculated on day 15 of incubation by puncturing with a sterilized egg shell boring needle. Honey supplementation was done with the help of disposable 24G needle into the egg yolk sac and were sealed immediately with molten paraffin. For *in ovo* injection honey dose was adopted from a recent study (Abdullah et al., 2018). All the eggs were incubated according to standard hatchery practices (99.9 to 100.0°F; 60 to 70% humidity) up to 21 d.

Post-hatch rearing of chicks

On day 1 post-hatching, the chicks of both groups were transferred to farm to evaluate the growth performance. A floor space of ½ sq. ft. per broiler was provided during the brooding period, while 1 sq. ft. per broiler during the later stage of rearing. The chicks were reared for six weeks of age, and supplied with a standard commercial broiler feed. Both temperature and humidity were maintained according to standard farming conditions. Feed and water were provided *ad libitum*. Body weight and feed consumption were recorded on daily basis that were used to calculate the feed conversion ratio (FCR). On day 21 and 42, ten chicken from each group were randomly selected, weighed and humanly slaughtered. Hot carcass weight was measured that was used to calculate carcass percentage.

Statistical analysis

The data was collected and analyzed using the student T-test through JMP statistical package software (version 5.0.1.a, SAS Institute Inc., Cary, NC). Significance level was determined at $P < 0.05$. All the results were presented as mean and pooled standard error of means (mean ± SEM).

RESULTS AND DISCUSSION

Hatchability percentage

There are various aspects that may affect hatchability of chicks such as availability of nutrient inside the egg, egg turning, and relative position of egg during incubation (Uni and Ferket, 2004). Moreover, Cardeal et al. (2015) and Ingram et al. (1997) determined that hatching process requires sufficient amount of energy, and *in ovo* supplementation provide extra energy to the chick to hatch out properly, thus leading to greater hatching percentage. In present study, eggs treated with *in ovo* honey showed a significantly ($P < 0.05$) higher hatchability percentage as compared to saline treated eggs (control group; Table 1). As compared to control group (75.0±0.43) honey inoculated group (87.5±0.25) exhibited a 16.67% increase in hatchability percentage. Similar findings were also reported by Ohta and Kiddt, (2001), Bhanja and Mandal, (2005), Tako et al. (2004), Smirnov et al. (2006), Hajati et al. (2014) and Yair et al. (2013) who examined higher

hatching percentage and better post hatch performance through *in ovo* supplementation of nutrients like carbohydrate, amino acid, vitamin C, minerals like Zn, Mn Cu, P, K, Na and grape seed extract. Positive effects of *in ovo* feeding on hatchability were also been reported by Uni et al. (2005) and Ferket, (2006) in their studies.

Table 1: Effects of *in ovo* honey administration on the hatchability and initial birth weight of chicks.

Parameter	Groups*		*P* value
	Control	Honey treated	
Hatchability (%)	75.0±0.43[b]	87.5±0.25[a]	0.016
Birth weight (g/bird)	44.5±0.33[b]	47.0±0.45[a]	0.046

*Results were presented as mean ± standard error of mean

[a-b] Means in a row not sharing common superscript letter are significantly different.

Carbohydrates (2.5% maltose + 2.5% sucrose) injection in pigeon's eggs during embryonic development (*in ovo*) is known to improve ($P < 0.05$) the hatching percentage from 82.50% to 88.75% (Dong et al., 2013). Noy and Sklan, (1998) reported that the chicken embryo has limited energy resources in amniotic fluid and the yolk sac. Honey is a rich energy source containing many nutrients viz., carbohydrates, amino acids, vitamins, and minerals (USDA nutrients database, 2014), that could be potential cause of hatchability improvement as observed in in present study. It is well established that scarcity of nutrients during late incubation period may be overcome by *in ovo* supplementation that result improved hatching and growth as well (Foye et al., 2006; Luqman et al., 2021). Further *in ovo* studies should be warranted using individual honey components (carbohydrates, amino acids, vitamins, minerals etc) to investigate which honey constituent influenced the physiological process of hatchling and resulted in improved percentage of hatching.

Initial birth weight

In current study, weight of newly hatched chicks is shown in Table 1. Results showed that *in ovo* honey treated group (47.0±0.45) has a 5.6% higher ($P < 0.05$) birth weight as compared to control group (44.5±0.33). In some other *in ovo* trials, higher birth weights were observed after administration of nutrients like vitamins, carbohydrates and trace elements (Foye et al., 2006; Uni et al., 2005; Bhanja and Mandal, 2005). The increase in birth weight after *in ovo* supplementation indicated the availability of additional energy required to complete the incubation period. This additional energy source probably supported the late-term development of the embryo, resulting in a significant increase in body weight of the day old chicks (Uni et al., 2005).

Dong et al. (2013) reported that *in ovo* injection of carbohydrates (mixture of 2.5% maltose + 2.5% sucrose) significantly improved the body weight of pigeons at hatch from 15.56g to16.45g. Similarly, in a recent study, coenzyme Q10 was known to improve body weight of chicks, up to 4.74% increase at hatching (Kalantar et al., 2019).

Studies suggested that hatching weight is a major indicator of chickens marketing. Wilson, (1991) stated that each gram of increase in body weight at hatching leads to 8 to 13 g of body weight at marketing age. While Uni et al. (2005) reported that each gram of increased hatching weight due to *in ovo* feeding leads to a 25 to 30 g increased weight gain at d 25 in broiler chicks. From our results and that of aforementioned researchers, it could be suggested that *in ovo* supplementation is a useful tool to get the market weight of poultry in least time.

Post hatch performance

Results presented in Table 2 showed that in 1st week, *in ovo* honey treated group exhibited higher ($P < 0.05$) body weight as compared to control group. While FCR and mortality were significantly ($P < 0.05$) improved and reduced, respectively, in honey treated broilers compared to the control group. In 2nd week, body weight and feed intake were significantly improved ($P < 0.05$), while FCR and mortality were significantly ($P < 0.05$) improved in honey treated group as compared to control group. In 3rd week, body weight and FCR were significantly improved ($P < 0.05$) by supplementation of *in ovo* honey as compared to control group. In 4th and 5th week, body weight and feed intake were significantly ($P < 0.05$) improved in honey inoculated group and compared to saline inoculated group (control). In 6th week, body weight and FCR were significantly improved in honey treated group as compared to control group. These results are in agreement with the findings of a previous study who reported that *in ovo* feeding of the exogenous nutrients increase the intestinal development thus improved villi size may help in improving the digestion, which ultimately results improved weight gain, feed intake and better FCR (Kornasio et al., 2011).

Results of overall performance showed that *in ovo* honey inoculation remarkably improved ($P < 0.05$) the performance of broilers by improving weight gain, feed intake, FCR and lowered ($P < 0.05$) the mortality as compared to saline treated (control) group broilers (Table 2). These results are in accordance with a recent study of Kalantar et al. (2019) who reported that *in ovo* inoculation of Q10 at the rate of 0.1 to 0.2 ml per egg significantly improve the post hatch performance of broilers at 21d, 42d and 1-42d. The authors reported 5.63 to 6.84g increase in weight gain at 21d and 5.63 to 7.73g increase in weight gain at 42d by the inoculation of Q10 coenzyme. Similarly, 0.11 and

Table 2: Effects of *in ovo* honey administration on the performance of broiler chickens.

Parameters	Groups*		*P* value
	Control	Honey treated	
1st week			
Body weight (g/bird)	111±1.67[b]	145±1.82[a]	0.030
Feed intake (g/bird)	208±2.76	210±3.07	0.119
FCR (g/g)	1.87±0.04[a]	1.45±0.3[b]	0.021
Mortality	5.00±0.00[a]	2.00±0.00[b]	0.004
2nd week			
Body weight (g/bird)	201±2.19[b]	244±2.95[a]	0.017
Feed intake (g/bird)	394±3.86[b]	411±3.55[a]	0.048
FCR (g/g)	1.96±0.07[a]	1.60±0.05[b]	0.027
Mortality	2.00±0.00[a]	0.00±0.00[b]	0.000
3rd week			
Body weight (g/bird)	268±2.19[b]	302±3.16[a]	0.022
Feed intake (g/bird)	539±7.30	555±8.04	0.083
FCR (g/g)	2.05±0.02[a]	1.84±0.02[b]	0.032
Mortality	0.00±0.00	0.00±0.00	-
4th week			
Body weight (g/bird)	325±3.22[b]	369±4.05[a]	0.018
Feed intake (g/bird)	634±5.66[b]	724±6.11[a]	0.030
FCR (g/g)	1.95±0.01	1.96±0.02	0.107
Mortality	0.00±0.00	0.00±0.00	-
5th week			
Body weight (g/bird)	387±3.99[b]	442±4.46[a]	0.009
Feed intake (g/bird)	782±6.89[b]	920±8.00[a]	0.026
FCR (g/g)	2.02±0.03	2.08±0.02	0.099
Mortality	0.00±0.00	0.00±0.00	-
6th week			
Body weight (g/bird)	456±5.77[b]	490±4.08[a]	0.021
Feed intake (g/bird)	985±9.95	1011±9.98	0.064
FCR (g/g)	2.16±0.02[a]	2.06±0.01[b]	0.044
Mortality	0.00±0.00	0.00±0.00	-
Overall			
Body weight (g/bird)	1748±15.03[b]	1992±20.51[a]	0.038
Feed intake (g/bird)	3542±22.93[b]	3831±29.39[a]	0.029
FCR (g/g)	2.00±0.00[a]	1.85±0.01[b]	0.037
Mortality	7.00±0.00[a]	2.00±0.00[b]	0.001

*All results were presented as mean ± standard error of mean.

[a-b] Means in a row not sharing common superscript letter are significantly different.

0.08 percent increase in FCR was recorded at 21 and 42d respectively as compared to control group. Also, improved performance of pullets (Bhattacharyya et al., 2018) and broilers (Abdullah et al., 2018) was reported in post hatch period resulting from *in ovo* injection of nutrients during embryonic life.

Results regarding effects of *in ovo* honey inoculation on carcass weight and dressing out percentage of broilers have been presented in Table 3. *In ovo* honey inoculation significantly ($P < 0.05$) improved the live weight and hot carcass weight at both 21 and 42 day as compared to control. However, it has non significant effects ($P > 0.05$) on the dressing percentage of broilers on day 21 and 42 (Table 3). In contrast to these results another study reported significant effects of *in ovo* honey administration on carcass

Table 3: Effects of *in ovo* honey administration on carcass weight and dressing percentage of broiler chickens.

Parameters	Groups*		*P* value
	Control	Honey treated	
21 day			
Live weight (g)	567±6.19[b]	680±8.06[a]	0.034
Hot carcass weight (g)	399±4.99[b]	491±7.20[a]	0.037
Carcass (%)	70.4±1.04	72.2±1.8	0.071
42 day			
Live weight (g)	1761±16.17[b]	1998±17.09[a]	0.026
Hot carcass weight (g)	1241±10.82[b]	1452±13.43[a]	0.036
Carcass (%)	70.5±0.07	72.7±0.08	0.055

*Results were presented as mean ± standard error of mean.

[a-b] Means in a row not sharing common superscript letter are significantly different.

weight of broilers at market age (Abdullah et al., 2018). Previous researches reported that the degree of response to *in ovo* injection solely depend on egg size, genetics and age of parent stock, and incubation conditions like disinfection e.t.c (Uni and Ferket, 2004; Salary et al., 2014).

CONCLUSIONS

From the results of current investigation, it could be concluded that *in ovo* supplementation of honey had significant positive effects on hatchability, hatching weight and post hatch performance of broiler chickens. Results also shows that honey inoculation is safe as it has no negative effect on hatching, as well as early chick mortality.

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CONFLICT OF INTEREST

There are is conflict of interest to declare.

AUTHORS CONTRIBUTION

SSM carried out the experiments; AAK conceived the experiment; FMK and KKM helped in data.

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