Effect of *in ovo* injection of glucose on growth, immunocompetence and development of digestive organs in turkey poults

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Three hundred fertile eggs of small white turkey were divided into 7 groups. First four groups were injected with 1 ml 10% glucose on 21st and 25th day of incubation through 25mm needle at broad and narrow end of egg. Fifth and sixth group was selected as 21st and 25th day sham control (1 ml sterile water), respectively. Seventh group was maintained as un-injected control. *In ovo* injection of glucose on 21st day resulted the deposition in allantoic cavity and yolk sac, whereas, on 25th day deposition sites were amniotic sac and yolk sac. Percent hatchability on fertile egg set basis was higher in 25th day sham control group (90.9) and un-injected control (87.5) whereas, glucose injection on 25th day into yolk sac (73.7) and amniotic sac (72.2) had lower hatchability. Overall, glucose injection on 25th day had better hatchability than 21st day injection. Chick weight to egg weight ratio was significantly (P<0.001) higher when glucose was deposited either in the yolk sac (65.11%) or in amniotic sac (64.27%) than un-injected control (62.27%). Glucose deposition in yolk sac had numerically higher body weight through out the experimental period and at 6 weeks of age there was difference of 76-78 g in body weight between control and yolk sac group. Humoral immune response was significantly higher (P< 0.001) in chicks hatched from eggs injected with glucose either on 21st day or 25th day of incubation than the control group (8.08 vs 5.50). Glucose injected in to the yolk sac on 21st d had higher titre value than other glucose injected groups. Higher bursa weight was noted in *in ovo* glucose injected groups than control (188 vs 162 mg %). Cell-mediated immune response (*in vivo* PHA-P response measured as footpad index) was not affected by glucose treatment. *In ovo* injection of glucose resulted in significantly (P< 0.05) lower liver weight as compared to control. Hence, it may be concluded that *in ovo* injection of 10% glucose on 21st day in to the yolk sac of developing embryo may enhance post hatch turkey poult growth and also may elicit better humoral immune response.

Key words: *In ovo* injection; glucose; hatchability; immunity; turkey poults

Introduction

Maintenance of glucose homeostasis during few days pre and post hatch is a great challenge in a chick's life. The primary source of glucose needed for hatching activities is the liver and gluconeogenesis from protein of amnion and muscle. Glycolysis rather than fatty acid oxidation is needed at hatching to provide energy as oxygen supply is limited during the transition from chorioallantois to pulmonary respiration (Hoiby *et al.,* 1987). At day of hatch glycogen stores decrease substantially, and remain low until the newly hatched chick has full access to oxygen, necessary to mobilize and utilize body fat reserves and assimilate external diet (Hazelwood, 2000). Just after hatch, the chick undergoes rapid physical and functional development of the gastrointestinal tract for digestion and assimilation of nutrients. However in most commercial operations, the chicks get access to feed several hours after hatching. This may decrease hatchling weight and immunity of chicks. These limitations in early energy status may be alleviated by administering refined carbohydrates or amino acids into the amnion or yolk sac (*in ovo* feeding) at different days of incubation, thereby improving chick livability and growth (Uni and Ferket, 2004; Bhanja *et al*., 2004). It has already been noted that glucose added to drinking water helps to suppress gluconeogenic enzyme activity (Donaldson, 1995). The digestive tract of the hatchlings has limited ability for digestion and utilization of diet rich in proteins and carbohydrates (Uni and Ferket, 2004). Hence, early access to highly digestible feed is beneficial but the benefits are solely dependent on the initiation of feed consumption unlike *in ovo* feeding. It has been observed that in ovo feeding of carbohydrates into the amnion increase hatchling weight in broilers and turkeys (Ferket and Uni, 2002). Further, *in ovo* feeding of carbohydrates also enhances enteric development (Ferket and Uni, 2002). Owing to the importance of *in ovo feeding* and its role in improving the hatchling weight, a study was undertaken to examine the effect of *in ovo* injection of glucose on the growth, immunocompetence and development of digestive organs in turkey poults.

Materials and methods

Fertile eggs (n=300) of small white turkey breeder hens maintained on adequate nutritional plane were collected, fumigated, weighed, divided into 7 groups and placed group wise in a forced draft incubator. First four groups were injected with 1 ml 10% glucose on 21^{st} and 25^{th} day of incubation through 25mm needle at broad and narrow end of egg. Fifth and sixth groups were selected as 21^{st} and 25^{th} day sham control (1 ml sterile water), respectively. Seventh group was maintained as un-injected control. The injection was carried out under laminar flow system where the temperature of the chamber was maintained at 35^{0}C. The *in ovo* injection was done through a pinhole made at the respective end of the egg and was completed within 30 minutes. Immediately after the injection, the site was sealed with sterile paraffin and eggs were returned to the incubator. One control group, which did not receive any injection was was kept in laminar flow system for 30 minutes to equate the injectable environment. Sterile water injection was included as sham control, primarily to rule out a possible negative response caused by the stress of injection and handling. On 25^{th} d the eggs were shifted to the hatcher and kept in the respective pedigree hatching boxes. On the day of hatch chicks were weighed, wing banded and transferred to the battery brooders for growth performance study. The chick weight to egg weight (pre-incubated) ratio and hatching percentages were compared among treatment groups to see the effect of glucose injection. The chicks hatched from the respective treatment group were distributed in 4 tier battery brooder cages having thermostatic control of temperature with provisions for separate feeder, waterer and droppings trays. The chicks hatched from the above experiment were fed ration having 28% CP and 2800 ME/kg up to 6 weeks of age. At 4 weeks of age 6 birds from each treatment group were taken to determine important immunocompetent traits such as antibody response to 1% sheep red blood cells (Siegel and Gross, 1980) and cell mediated immune response to PHA-P (1mg PHA-P/ml of PBS) measured as foot web index (Corrier and De Loach, 1990). 6 birds from each treatment group were sacrificed to study the growth of different digestive organs (liver without gall bladder, proventriculus, gizzard, caeca, and small intestine) and immune organs (bursa, thymus and spleen) and expressed as g/100g of live body weight. All the data obtained were analyzed as per the standard statistical procedure (Snedecor and Cochran, 1980). Significant differences among treatment means were calculated as per DMRT test (Duncan, 1955).

Results and discussion

In ovo injection of glucose on 21^{st} day resulted the deposition in allantoic cavity and yolk sac, whereas, on 25^{th} day deposition sites were amniotic sac and yolk sac. Percent hatchability on fertile egg set basis was higher in 25^{th} day sham control group (90.9) and un-injected control (87.5) whereas, glucose injection on 25^{th} day into yolk sac (73.7) and amniotic sac (72.2) had lower hatchability. Overall, glucose injection on 25^{th} day had better hatchability than 21^{st} day injection *(Table 1).*

Table 1 Effect of *in ovo* injection of glucose on different days and deposition site on percent hatchability

Days	Deposition site	Percent Hatchability
21st	Allantoic Cavity	62.67
21st	Yolk Sac	56.75
25th	Amniotic Sac	72.22
25th	Yolk Sac	73.68
21st	Sham Control	76.00
25th	Sham Control	90.91
Un-injected Control		87.5
Day of injection		
21st		63.5
25th		75.76

Though there was no significant difference in chick weight and egg weight, but their ratio was significantly (P<0.001) higher where glucose was deposited either in the yolk sac (65.11%) or in amniotic sac (64.27%) than un-injected control (62.27%) *(Table 2)*.

Glucose deposition in yolk sac had numerically higher body weight through out the experimental period and at 6 weeks of age there was difference of 76-78 g in body weight between control and yolk sac group *(Table 3)*.

Table 2 Effect of embryonic age and site of *in ovo* injection on the turkey chick weight

Group	Egg Weight (g)	Chick Weight(g)	Ratio(%)
21d 25mm broad Allantoic	79.04±0.69	49.39^{ab}±0.58	62.46^{b}±0.41
21d 25mm narrow yolk	78.04±1.13	50.84^{b}±0.96	65.11^{c}±0.67
21d 25mm broad sham	79.34±1.05	49.71^{ab}±0.71	62.69^{b}±0.67
25d 25mm broad amniotic	79.45±0.6	51.05^{b}±0.47	64.27^{c}±0.35
25d 25mm narrow yolk	79.28±0.96	47.64^{a}±0.73	60.08^{a}±0.54
25d 25mm broad sham	78.37±0.84	49.11^{ab}±0.71	62.65^{b}±0.53
Control	79.70±0.89	49.66^{ab}±0.77	62.27^{b}±0.47
Sig Level	NS	P<0.006	P<0.001

Table 3 Effect of site of deposition on the body weight of turkey poults

Site of Deposition	BW 2nd wk (g)	BW 4th wk (g)	BW 6th wk (g)
Allantoic Sac	172.5	544.2	876.2
Yolk Sac	202.1	623.4	1022.6
Amniotic Sac	193.7	583.6	948.4
Uninjected control	190.4	594.3	945.7
Sig Level	NS	NS	NS

Humoral immune response measure as haemmaglutination titre (log 2) value in response to 1% sheep red blood cells was significantly higher (P< 0.001) in chicks hatched from eggs injected with glucose either on 21st day or 25th day of incubation as compared to the control group (8.08 vs 5.50).

Table 4 Effect of *in ovo* injection of glucose on SRBC and PHAP response

Treatments	HA Titre	PHAP
Glucose	8.08^{b}±0.380	0.450±0.035
Sham Control	5.50^{a}±0.51	0.576±0.128
Control	5.50^{a}±0.33	0.668±0.197
Sig Level	P<0.001	NS

Further it revealed that glucose injected in to the yolk sac on 21st d had higher titre value than other glucose injected groups. Cell-mediated immune response (*in vivo* PHA-P response measured as footpad index) was not affected by glucose treatment *(Table 4 & 5)*.

Apparently, a higher bursa weight was also noted in the *in ovo* glucose injected group than control (0.188 vs 0.162 mg %). *In ovo* injection of glucose resulted in significantly (P< 0.05) lower liver weight as compared to control.

Table 5 **Effect of embryonic age and site of *in ovo* injection on the HA titre and PHAP response**

Group	Anti SRBC titre (log 2)	PHAP Response
21d 25mm broad Allantoic	8.62^{b}±0.57	0.433±0.06
21d 25mm narrow yolk	8.75^{b}±0.98	0.350±0.046
21d 25mm broad sham	5.67^{a}±1.022	0.514±0.150
25d 25mm broad amniotic	7.50^{ab}±0.69	0.516±0.067
25d 25mm narrow yolk	6.60^{ab}±0.82	0.432±0.117
25d 25mm broad sham	5.40^{a}±0.40	0.638±0.197
Control	5.50^{a}±0.33	0.668±0.197
Sig Level	P<0.004	NS

Table 6 Effect of *in ovo* injection of glucose on the development of immune and digestive organ

Treatments	BURSA	THY	SPLEEN	LIVER	PANCREAS	PROV	GIZ
Glucose	0.188	0.119^{b}	0.142	1.776^{a}	0.301	0.358	2.167
Sham control	0.162	0.067^{a}	0.139	1.866^{ab}	0.300	0.410	2.218
Control	0.160	0.133^{b}	0.162	2.004^{b}	0.345	0.409	2.395
Sig Level	NS	P<0.02	NS	P<0.05	NS	NS	NS

Hence, it may be concluded that 10% glucose can be *in ovo* injected on 21st day when the site of deposition is yolk sac to enhance growth of turkey chicks and elicit better humoral immune response.

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