



**INFLUENCE OF *IN OVO* FEEDING WITH VITAMIN C OR  
GLUCOSE ON POST-HATCH PRODUCTIVE PERFORMANCE OF  
DANDRAWI CHICKS**

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**ABSTRACT:** A study was carried out to evaluate the effect of *in ovo* feeding during early-embryonic life with either vitamin C or glucose on hatchability, post hatch performance, carcass traits and gut histology. Two sets of Dandrawi fertile eggs (400 eggs each with average weight  $51.1 \pm 0.2$ g) were used in two separate trials. On d 14 of incubation, eggs of the first set (Trial 1) were examined and those containing live embryos were divided equally into 4 treatments (100 eggs/ treatment) and injected in the amnion as follows: T1 (negative control, no injection); T2 (positive control, injected with 0.1ml saline solution 0.9g/L); T3 (injected with 0.1ml 2.5 % water solved vitamin C); T4 (injected with 0.1ml 5% water solved vitamin C). Eggs of Trial II: T1 (negative control, no injection); T2 (positive control, injected with 0.1ml saline solution 0.9g/L); T3 (injected with 0.1ml 5 % water solved glucose); T4 (injected with 0.1ml 10% water solved glucose). Hatched chicks from each trial were allocated into 3 replicates/ treatment and brooded till 10 weeks old. Performance of chicks of T4 (Trial 1) hatched from the eggs injected with 5% vitamin C showed significant ( $P < .01$ ) improvement in BW, BWG, FCR and scored the lowest percent of mortality. However; measurements of carcass traits showed no significant differences among treatments. Furthermore, blood parameters were all within normal physiological values. Histological examination of the gut revealed positive influence ( $P < .01$ ) of 5% vitamin C injection level on height and width of duodenum villi. Results of the second trial showed significant ( $P < .01$ ) enhancement in performance of the chicks hatched from the eggs injected with 10% glucose. Also, histological evaluation of the same group scored the highest ( $P < .01$ ) values of duodenum villi height and width. In conclusion, *in ovo* injection at d 18 of incubation with either 0.1 ml 5% vitamin C or 0.1ml 10% glucose improved post hatch Dandarawi chicks' performance and gut histological traits related to nutrient absorption.

**Keywords:** In Ovo feeding, vitamin C, glucose, hatchability, performance, gut histology

## **INTRODUCTION**

Chicken eggs are precocial in a common sense that hatched chicks are in advanced state and skillful of movable and feeding on its own. The subsequent development of avian embryos and hatched chicks are influenced by the yolk nutrient status (Al-Murrani, 1982). Numerous nutrients have important structural, physiological, and immunological roles in avian embryogenesis and growth performance, arise during hatchability of eggs. *In ovo* nutrient administration may afford poultry enterprise with alternative method to improve hatchability rate (Ohta *et al.*, 2001). Progressing technology use of *in ovo* injection has become a “hot spot” in research at present.

First *in ovo* injection was used for the vaccination purpose for Marek’s disease (MD) at 18<sup>th</sup> day of incubation and observed better immunization (Sharma and Burmester, 1982). Beyond vaccines, several nutrients or compounds can be provided to the developing embryo via this route of administration.

Fresh laid eggs do not contain ascorbic acid (AA) which is synthesized in the embryo by means of egg yolk sac membrane at the first stage of embryo development (Yew, 1985). During the second half of the incubation period, the egg needs to dissipate heat to the environment of the incubator due to increased embryonic metabolic rate and heat production (French, 1997). Furthermore, incubator temperature has a subsequent influence on embryo development, hatchability, and post hatch performance (Lourens *et al.*, 2005, 2007, 2011; Barri *et al.*, 2011). Vitamin C is known as anti-stressor factor that boost the

performance of the bird (Pardue and Thaxton, 1986; Mahmoud *et al.*, 2004). It is possible that *in ovo* injection of vitamin C may be beneficial to embryos under thermo neutral or heat-stress conditions pre hatch. Subsequently, improved performance of birds post hatch (Zakaria and Al- Anezi, 1996; Ghonim *et al.*, 2009; Mohammed *et al.*, 2011 and Nowaczewski *et al.*, 2012).

Chicken eggs are rich in protein and lipids but poor in carbohydrates (Burley and Vadehra, 1989). Total carbohydrate in egg is less than 1% of the total nutrients and only 0.3% in free glucose form (Campos *et al.*, 2011). Therefore, carbohydrates in eggs may not be sufficient to meet the immediate metabolic demands of the embryo. *In ovo* injection has been proposed as a possible means of supplying exogenous nutrients to the amnion of the late-term avian embryo (Uni and Ferket, 2003; Uni *et al.*, 2005; Zhai *et al.*, 2011; Oliveira *et al.*, 2015). The nutrients within the egg provide energy and building blocks required for the metabolic needs of the growing embryo during its normal 21-day incubation (Foye *et al.*, 2006). Glucose is mainly stored as glycogen in the liver and in glycolytic muscles. Therefore, the stored glycogen is depleted during the hatching process (Uni *et al.*, 2005). The energy status or body weight of hatchlings is improved in response to the *in ovo* injection of carbohydrate solutions, including glucose, sucrose, maltose, or dextrin, singly or in combination with other nutrients (Tako *et al.*, 2004; Uni *et al.*, 2005; Zhai *et al.*, 2011). In a commercial scale *in ovo* fed chicks can open a new marketing strategy in future with different cost for distinct feed supplements (Loksha *et al.*, 2017).

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Therefore, the aim of our study was to evaluate the effect of *in ovo* feeding in early embryonic life with either vitamin C or glucose on hatchability, post hatch performance and gut histological examination.

### **MATERIALS AND METHODS**

**Experimental design.** Two sets of Dandrawi fertile eggs (400 eggs each with average weight  $51.1 \pm 0.2$ g) were collected from 12 months- old Dandrawi hens and used in two separate trials. On d 14 of incubation, all eggs were examined to identify and remove clear eggs and dead embryos, whereas those containing live embryos were divided equally into 4 treatments (100 eggs/ treatment). Eggs of Trial 1 were injected in the amnion on d 18 as follows: T1 (negative control, no injection); T2 (positive control, injected with 0.1ml saline solution 0.9g/L); T3 (injected with 0.1ml 2.5 % water solved vitamin C); T4 (injected with 0.1ml 5% water solved vitamin C). Eggs of the second set (Trial II) were treated in a similar manner to those of Trial 1 and injected in the amnion on d 18 as follows: T1 (negative control, no injection); T2 (positive control, injected with 0.1ml saline solution 0.9g/L); T3 (injected with 0.1ml 5 % water solved glucose); T4 (injected with 0.1ml 10% water solved glucose).

**Injection procedure.** On day 18 of incubation, eggs were removed from the incubator to determine the injection site. The injection site was cleaned with 70% ethanol. Sharp dissection probe was dipped in ethanol to be sterilized and used to make a small puncture in egg shell. Insulin syringe (27gauge x 1/2" 13 nm) was used to administer all injections. The needle was

fully inserted through the hole created by the dissection probe and the contents of syringe were injected into the amnion of the egg shown in Figure (1). The site of injection was then sealed using liquid paraffin wax to prevent contamination. After completion of the injection, all eggs were returned to the hatcher.

**Management and diet.** The eggs were incubated at temperature 99°F and relative humidity 60% in Petersim incubator. At 14<sup>th</sup> day of incubation, all eggs were candled, and the infertile ones or those containing early dead embryos were removed and recorded. Consequently, at 18<sup>th</sup> day, eggs were transferred into hatcher Petersim at temperature 97 °F and relative humidity 65-70%. Hatched chicks were weighed, wing banded and reared under stander protocol of managerial and hygienic conditions of animal welfare. All chicks were brooded on floor pen covered with saw dust litter depth 2.5 Cm. The indoor temperature was kept at 33 °C for the first week of age then declined gradually by 2.8 degree weekly till constant at 24 °C during the rest of the experiments. The artificial lighting was provided continuously for 24 hour / day. Cleaned water was available all the time and feed was offered *ad libitum* as mash. Diet was formulated to meet all nutrients requirements according to NRC (1994) shown in Table (1)

### **Parameters measured.**

**Fertility & hatchability.** At the end of hatching, fertility % and hatchability% were calculated according to the equations:

$$\text{Fertility \%} = \frac{\text{total eggs} - \text{clear eggs}}{\text{total eggs}} \times 100$$

$$\text{Hatchability\%} = \frac{\text{total hatched healthy chicks}}{\text{total fertile eggs}} \times 100$$

Growth performance. Body weight was recorded biweekly to the nearest gram from hatch till 10 week-old and body weight gain was calculated according to the equation:

BWG = final body weight – initial body weight. Feed consumption was recorded biweekly at the same time of recording body weight and feed conversion was calculated according to the following equation:  $\text{FCR} = \frac{\text{feed consumption(g)}}{\text{body weight gain (g)}}$

Carcass characteristics. At the end of each trial 10 week –old, a total number of 24birds (6 birds/ treatment) around average body weight of the treatment were fasted for 8 hours before slaughter. The birds were weighed individually as pre-slaughter weight. Then, birds were slaughtered by cutting the jugular vein, when complete bleeding was achieved; slaughter weight was recorded and then the birds were scalded and plucked to remove the feather. After plucked the carcass was opened and edible and non-edible organs were removed to obtain the dressed carcass weight. Carcass percent was calculated as a percentage of the live body weight. Also, liver, spleen, gizzard and bursa were weighted and expressed as % of live weight. Length of esophagus, cecum and rectum were recorded. Moreover, length and weight of small intestine (duodenum-jejunum-ileum) were measured.

*Blood parameters.* At slaughtering, blood samples were collected from 24 birds /trial

(6/treatment) in non- heparinized tubes and centrifuged (3000 rpm) for 15 minutes to obtain blood serum. Serum samples were collected and kept frozen at -20°C until being analyzed. The obtained serum samples were analyzed colorimetrically by using kits purchased from El-Gomhorya Company, Egypt. Glucose, total protein, albumin, globulin, triglycerides, calcium and phosphorus were determined. Serum globulin values were calculated by subtracting albumin values from their corresponding total proteins values of the same sample, and albumin / globulin ratio was calculated.

*Histological studies.* At the slaughter, sample from 18 birds / each trial were taken to determine the histological studies. Therefore, a small part of duodenum, jejunum and ileum were taken and kept in formalin solution 10%. Three sections of the small intestine parts (duodenum-jejunum- ileum) were prepared using standard paraffin embedding procedures by sectioning at 5 µm thicknesses, and staining with hematoxylin and eosin then mounted onto glass slides and observed under light microscope. Villus height and width, crypt depth and villus height/crypt depth were measured using an image processing and analyzing system (Image-J), and were expressed in micrometers (µm). Measurements of villus height and crypt depth were taken only from sections where the plane of section ran vertically from the top of villus to the base of an adjacent crypt (Jawad *et al.*, 2016). Values presented are meant from seven samples of villi measured from the Tip to the crypt mouth and seven associated crypts measured from

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the crypt mouth to the base (Xu *et al.*, 2003).

**Statistical Analysis.** Data of growth performance were statistically analyzed using Analysis of Variance (ANOVA), applying the General Linear Model (GLM) Procedure, SAS Software, Version 9.2 (SAS, 2009). Percentage values were transformed using arcsine before statistical analyses. Significant differences among treatment means were separated by Duncan's multiple range test (Duncan, 1955) with a 5% level of probability. All data obtained within each experiment were analyzed using the following Model:

$Y_{ijk} = \mu + T_i + E_{ijk}$ , where  $Y_{ijk}$  = observation,  $\mu$  = the overall mean,  $T_i$  = the effect of treatment,  $i$  (1- 4),  $E_{ijk}$  = experimental random error

### **RESULTS AND DISCUSSION**

Trial 1: Effect of *in ovo* injection with different levels of vitamin C on :

**1.Chicks' performance:** Results of BW, BWG, FC, FCR and mortality % are presented in Table (2). At the end of the trial (10 week-old), significant improvement ( $P < .01$ ) in BW in group injected with 5% vitamin C and was superior to all other treatments ; control, saline, and 2.5% C ( $599.7 \pm 9.2$  vs.  $568.4 \pm 11.5$ ,  $552.6 \pm 10.6$ ,  $543.6 \pm 9.2$ g; respectively). Similar trend was found in the overall BWG. Feed consumption during the period 0-10 weeks of age indicated significant lower FC in all injected treatment ( $P < .01$ ) compared to the control group. Moreover, FCR recorded best result ( $P < .01$ ) in group injected with 5% C ( $3.2 \pm .05$ ). The positive effect of *in ovo* injection of vitamin C 5% on FCR may explain the improvement in feed utilization, hence,

better BW and BWG of the chicks in this treatment. Our results agreed with (Rizk and Ibrahim, 2014; Hajati *et al.*, 2014 and Al-Hassani and Alkafaje, 2015). In contrast, different study using broiler eggs (Zhang *et al.* 2018), or quail eggs (Babacanoğlu, 2018) injected with ascorbic acid claimed no effect on chicks performance.

Mortality recorded the lowest percentage (2%) in the treatment received 5% C injection compared to all treatments (7.9, 6.0, 8%; respectively). Results of hatchability of fertile eggs as affected by *in ovo* injection with vitamin C revealed no differences among treatments.

### **2.Carcass traits and digestive organs:**

The results of carcass traits, weight and length of digestive organs as affected by *in ovo* injection with different levels of vitamin C are presented in Tables (3) and (4). Results of carcass traits presented in table (3) revealed no significant differences among treatments in all parameters studied; dressed carcass weight and dressed carcass%.

Furthermore, absolute weight and % of liver, gizzard, spleen and bursa were not affected by *in ovo* injection with vitamin C. In respect to weight and length of digestive organs (Table, 4), it appeared that length of esophagus, duodenum, jejunum, ileum, cecum, rectum and duodenum weight were not affected by *in ovo* injection with vitamin C. However; inconsistent trend was found in jejunum and ileum weights of the injected treatments ( $P < 0.05$ ).

**3. Blood parameters:** Results presented in Table (5) indicated that the control group recorded the lowest values of all blood parameters among treatments. However, T3

showed the highest concentrations of T. protein, globulin, triglycerides and phosphorus. While, chicks in T4 counted the highest values of glucose, A/G ratio and cholesterol. It is worth noticing that *in ovo* injection of vitamin C stimulated chicks' immune response as indicated by increased levels of A/G and globulin in Dandrawi chicks. Our results are in agreement with those obtained by Rizk and Ibrahim (2014) and Badran et al., (2017).

**4. Histological traits of GIT:** Histological traits of small intestine (duodenum, jejunum and ileum) as affected by *in ovo* injection with vitamin C are presented in Table (6) and Figure (2). In this study, histological examination of the duodenum in the treatment injected with 5% vitamin C showed significant ( $P < .01$ ) increase in villi height, width and V/C ratio ( $1836.3 \pm 139.0$ ,  $160.6 \pm 9.1$  and  $3.5$ ; respectively) compared to the control group ( $1374.0 \pm 64.5$ ,  $121.8 \pm 17.2$  and  $2.9 \pm 0.26$ ; respectively). The administration of exogenous nutrients into the amnion enhanced the intestinal development favorably disposed towards the intestinal capacity to digest nutrients and may explain the improvements in BWG and FCR (Tako et al., 2004 and Feng et al., 2007). Maximum digestion and absorption are believed to occur as villus height to the crypt depth ratio increased (Chaing et al., 2010).

In jejunum sections, more positive influential effects of 2.5% and 5% treatments were observed on villi height; width and ratio of V/C. The microscopic structure of small intestine in terms of villus height and crypt depth is considered

the main indicator of intestinal development, health and functionality, resulting in efficient nutrient digestion and absorption in Dandrawi chicks (Jawad et al., 2016 and Naji et al., 2017).

Results of ileum histology (Table, 6) showed that villi height was sharply decreased ( $P < 0.01$ ) in the treatment injected with 5% vitamin C than the control ( $1151.7 \pm 28.2$  vs.  $1301.5 \pm 39.7$ ). It is worthy noticing that the duodenum showed greater villi height and crypts depth than the other gut segments and higher V/C ratio than the ileum (Biasato et al., 2018). Apparently, the small intestine represents the major site of nutrient absorption (Dingle, 1991) and the ileum has been suggested to be more inclined to bacterial colonization (Forder et al., 2007). Villi width was not affected by treatments. Similar trend of reduction in crypt depth was found ( $P < 0.01$ ) in the treatment injected with 2.5% vitamin C compared to the control ( $221.1 \pm 9.7$  vs.  $327.6 \pm 9.8$ ). Our data agree with those of Foye et al., (2007) who found that injecting eggs with nutrients makes digestive system capacity greater in digestion and absorption of nutrients more than non-injected eggs. Also, Tako et al. (2004) noticed that *in ovo* injection with nutrients increased surface area of jejunum villi by about 45% at hatch in comparison with non-injected control groups.

**Trial 2: Effect of *in ovo* injection with different levels of glucose:**

**1. Chicks' performance:** Growth performance of glucose injected groups on post hatch performance of Dandrawi chicks including BW, BWG, FC, FCR and mortality % during the period from day old till 10 weeks

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of age are presented in Table (7). Hatchability percentage showed high numerical values of all injected treatments compared to the control group. BW at 1 day- old revealed significant increase ( $P<.05$ ) in the treatment injected with 5% glucose compared to the control and those injected with 10% glucose, but was not different from the treatment injected with saline.

Remarkably, at the end of the trial (10- week old), BW of chicks received 10% glucose showed the highest of all treatments. Similar trend was recorded for BWG. Higher dose of glucose (10%) resulted in positive influential effect on chicks' performance than lower dose (5%). Furthermore, the introduction of external carbohydrates, as readily available energy sources, may help to spare protein and fatty acids that would normally be used for gluconeogenesis so that embryo growth may be optimized (Uni and Ferket, 2004; Uni *et al.*, 2005; Foye *et al.*, 2006, 2007; Bhanja *et al.*, 2008; Bottje *et al.*, 2010 and Salmanzadeh *et al.* 2012). Although, feed consumption was significantly decreased ( $P<.01$ ) in 5 and 10% glucose injected groups compared to the control and saline, yet improved FCR, was recorded for the glucose injected groups ( $3.4\pm 0.07$ ,  $3.2\pm 0.06$  vs.  $3.9\pm 0.07$  and  $3.6\pm 0.07$ ; respectively). Truly, rapid growth rate, better feed conversion ratio, and marketable body weight can be enhanced through the *in ovo* glucose feeding. In a commercial scale, *in ovo* feeding, chicks can open a new marketing strategy in future with different cost for distinct feed supplements (Loksha *et al.*, 2017). Concerning mortality, numerical lower percentage but not significantly different was observed in all injected groups compared to the control (6, 6 and 6 vs. 7.9, respectively).

### **2. Carcass traits and digestive organs:**

Results of carcass traits in Table (8) indicate no differences among treatments in dressed carcass and dressed carcass%. Also, absolute weights and percent of organs (Table, 9) were

not affected by *in ovo* injection treatments. However, duodenum length scored significantly ( $P<.05$ ) the highest in the treatment injected with 10% glucose among all treatments. Similarly, ileum weight was significantly the largest in the same treatment ( $P<.05$ ). Rizk and Ibrahim (2014) showed that *in ovo* injection of glucose had increased length of small intestine. This improvement in the development of gastro-intestinal tract plays an important role in the growth of chick during early stages as reported by Nir *et al.* (1996) due to immediate access to nutrients which further stimulated the production of digestive enzymes.

### **3. Blood parameters:**

Result of blood parameters Table, 10 showed no differences among treatments in glucose concentrations. On the contrary, T. protein, albumin and A/G levels were significantly ( $P<.01$ ) higher in all *in ovo* injected groups than the control. In addition, serum globulin was significantly higher in T3 and T4 but not different from saline group. Stimulatingly, *in ovo* injection of glucose roused chicks' immune response as indicated by increased levels of A/G and globulin in Dandrawi chicks. Moreover,

cholesterol level was significantly ( $P<.01$ ) higher in glucose injected groups; T3 and T4. Serum TG level in T4 was the highest of all groups ( $P<.01$ ) followed by T3. Calcium levels were not different, while phosphorus was significantly higher in T3. Our results were in partial disagreement to, (Salmanzadeh *et al.*, 2012; Refaie *et al.*, 2018) who stated that *in ovo* injection with glucose exhibited no differences in blood glucose, triglycerides and total cholesterol.

### **4. Histological traits of GIT:**

Data of histological traits of small intestine (duodenum, jejunum and ileum) as affected by *in ovo* injection with glucose levels are presented in Table (11) and Figure (3).

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Results of duodenum reveal that villi height scored the highest ( $P<0.01$ ) value in the treatment injected with 10% glucose by about 1.3X compared to the control. However, saline injected group was not different from both 5 and 10% injected group. Furthermore, villi width was superior ( $P<0.01$ ) in the treatments injected with 5% and 10% glucose by about 1.9X compared to control ( $196.8\pm 28.2$  and  $190.8\pm 15.1$  vs.  $98.5\pm 11.5$ , respectively).

Moreover, the lowest value of crypt depth was recorded in the control group. Also, the group injected with 10% glucose exhibited significantly ( $P<0.05$ ) increased V/C ratio than the groups injected with 5% glucose and saline ( $3.1\pm 0.22$  vs.  $2.5\pm 0.12$  and  $2.5\pm 0.07$ , respectively). Our results agree with those of Chen *et al.*, (2009) who found that *in ovo* injection of glutamine and carbohydrates into duck eggs had improved small intestine development, as reflected in the increase in weight.

Results of jejunum showed that villi height and width were increased ( $P<0.01$ ) in the groups injected with 5% and 10% glucose compared to the control group by 1.3 and 1.6X ; respectively. However, saline injected group was not different from the glucose injected groups. No differences were detected regarding crypt depth and ratio V/C among treatments. Our results are in agreement with those obtained by Uni and Ferket (2004) who reported that *in ovo* injection of 1 ml of saline containing

carbohydrate at 18 d of incubation significantly increased jejunum villus height by over 45% within 48 h after injection. Jia *et al.*, (2011) also reported that *in ovo* feeding of 200 g/l maltose solutions to the chicken embryo enhanced the absorption of nutrients and thus the development of the jejunum villus and finally the weight of hatchlings. After *in ovo* feeding, the gastrointestinal tract (GIT) of hatchlings becomes functionally similar to that of conventional 2 day old chicks offered feed immediately after hatch and helps in accelerating the enteric development for greater digestive and nutrient absorptive capacity (Uni and Ferket, 2004 and Uni *et al.*, 2005 ).

As it could be revealed from Table 11 and Figure 3, the ileum section showed absent effect on villi height and width and inconsistent trend in crypts depth or V/C. Smirnov *et al.*, (2006) concluded that the presence of CHO in the intestinal lumen of the chick embryo improved intestinal morphology and consequently nutrient absorption.

**IN CONCLUSION,**

*in ovo* injection of Dandrawi eggs with either 5% vitamin C or 10% glucose resulted in improved growth performance and developed small intestine structure and absorption during brooding period from hatch till 10 weeks of age. Therefore, it is suggested using *in ovo* injection at these levels without any adverse effect on growth performance.

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**Table (1):** Composition and analysis of the experimental diet

<b>Ingredient</b>	<b>%</b>
Yellow Corn	67.0
Soybean meal (44%)	26.3
Wheat bran	3.0
Limestone	1.0
Di calcium phosphate	2.0
DL-Methionine	0.1
NaCl	0.3
Premix*	0.3
Total	100
<b>Calculated values</b>	
ME (Kcal/Kg)	2876
Crude protein (%)	18.04
Crude fiber (%)	3.73
Crude fat (%)	2.85
Calcium (%)	0.97
Available phosphorus (%)	0.44
Methionine (%)	0.41
Lysine (%)	0.95
Sodium (%)	0.23
<b>Analyzed values</b>	
Crude protein (%)	18.69
Crude fiber (%)	4.07
Crude fat (%)	3.38
Crude ash (%)	5.73

\* Each kg of vitamin mineral premix: contains: vitamin A, 1200000; vitamin D3, 300000IU; vitamin E, 700 mg; vitamin K3, 500 mg; vitamin B1, 500 mg; vitamin B2, 200 mg; vitamin B6, 600 mg; vitamin B12, 3 mg; folic acid, 300mg; choline chloride, 1000 mg; Niacin, 3000 mg; Biotin, 6 mg; panathonic acid, 670 mg; manganese sulphate, 3000 mg; iron sulphate, 10000 mg; zinc sulphate, 1800 mg; copper sulphate, 3000 mg; iodine, 1.868 mg; cobalt sulphate, 300 mg; selenium, 108 mg.

**Table (2):** Effect of *in ovo* injection with vitamin C on chick's performance (g/bird)  $\bar{X} \pm SE$

Parameters	Treatments				Sig.
	Control <sup>1</sup>	Saline	2.5%vit.C	5%vit.C	
BW <sub>0</sub> <sup>3</sup>	31.5±0.4	32.4±0.3	32.7±0.4	32.6±0.4	NS
BW <sub>f</sub> <sup>3</sup> 10 wks	568.4 <sup>b</sup> ±11.5	552.6 <sup>b</sup> ±10.6	543.6 <sup>b</sup> ±9.2	599.7 <sup>a</sup> ±9.2	**
BWG 0-10 wks	536.7 <sup>b</sup> ±11.4	520.3 <sup>b</sup> ±10.5	511.0 <sup>b</sup> ±9.1	567.1 <sup>a</sup> ±9.2	**
FC 0-10wks	2047.3 <sup>a</sup> ±10.9	1840.2±14.8 <sup>c</sup>	1885.9 <sup>b</sup> ±10.3	1808.6 <sup>d</sup> ±3.4	**
FCR 0-10 wks	3.9 <sup>a</sup> ±0.07	3.6 <sup>b</sup> ±0.07	3.7 <sup>ab</sup> ±0.07	3.2±0.05 <sup>c</sup>	**
Mortality%	7.9	6.0	8.0	2.0	
Hatchability%	49.4	65.6	58.1	65.1	

<sup>1</sup> Total number of chicks control =38, saline =50, 2.5%vit.C = 50, 5% vit.C =50

<sup>2</sup> a, b, c means with the same superscripts within the same row are not significant

<sup>3</sup> BW<sub>0</sub> = body weight at day – old, BW<sub>F</sub> = body weight at 10 week- old

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.01)

**Table (3):** Effect of *in ovo* injection with vitamin C on carcass traits ( $\bar{X} \pm SE$ )

parameters	Treatments				Sig.
	Control	Saline	2.5%vit.C	5%vit.C	
Live weight (g)	550.1±20.4	562.1±25.8	540.1±20.9	566.1±26.6	NS
Dressed carcass, g	344.8±16.2	341.0±17.1	326.6±12.5	351.3±16.6	NS
Dressed Carcass, %	62.5±0.38	60.6±0.50	60.5±0.49	62.0±0.28	NS
Liver weight , g	13.7±0.50	14.9±0.85	14.4±0.48	14.1±0.74	NS
Liver, %	2.5±0.25	2.6±0.26	2.6±0.23	2.5±0.21	NS
Gizzard weight, g	16.0±0.86	16.6±1.08	15.5±0.86	16.0±0.56	NS
Gizzard, %	2.9±0.38	2.9±0.34	2.8±0.35	2.8±0.22	NS
Spleen weight, g	1.7±0.11	2.0±0.20	1.8±0.30	1.7±0.09	NS
Spleen, %	0.31±0.10	0.36±0.15	0.34±0.28	0.31±0.14	NS
Bursa weight , g	1.4±0.28	1.6±0.30	1.1±0.16	1.2±0.20	NS
Bursa,%	0.26±0.29	0.29±0.25	0.21±0.17	0.22±0.21	NS

NS= non significant

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**Table (4):** Effect of *in ovo* injection with vitamin C on weight and length of digestive organs ( $\bar{X} \pm SE$ )

parameters	Treatments				Sig.
	Control	Saline	2.5%vit.C	5%vit.C	
Esophagus length(cm)	16.0±0.75	17.2±0.68	16.1±0.54	17.0±1.1	NS
Duodenumlength(cm)	22.0±0.54	22.5±0.85	21.9±0.85	24.0±1.4	NS
Duodenum weight (g)	6.4±0.64	7.3±0.68	6.2±0.41	6.9±0.76	NS
Jejunum length (cm)	49.9±2.4	52.1±3.2	51.0±2.1	49.6±2.8	NS
Jejunum weight (g)	9.3±0.63 <sup>b</sup>	11.3±0.84 <sup>a</sup>	9.5±0.33 <sup>b</sup>	9.4±0.60 <sup>b</sup>	*
Ileum length(cm)	51.9±1.3	52.5±3.04	52.4±1.5	52.0±2.0	NS
Ileum weight (g)	6.5±0.26 <sup>b</sup>	7.7±0.50 <sup>a</sup>	7.2±0.23 <sup>ab</sup>	6.8±0.37 <sup>ab</sup>	*
Cecum length(cm)	23.5±0.76	23.0±0.77	24.0±1.05	24.5±0.81	NS
Rectum length(cm)	6.9±0.39	7.1±0.43	6.0±0.68	7.1±0.38	NS

<sup>a, b, c</sup> means with the same superscripts within the same row are not significant

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.01)

**Table (5):** Effect of *in ovo* injection with vitamin C on blood parameters ( $\bar{X} \pm SE$ )

parameters	Treatments				Sig.
	Control	Saline	2.5%vit.C	5%vit.C	
Glucose(mg/dl)	149.7 <sup>b</sup> ±4.3	158.8 <sup>ab</sup> ±5.1	167.8 <sup>ab</sup> ±5.7	169.0 <sup>a</sup> ±7.8	*
T. Protein(g/dl)	2.9 <sup>c</sup> ±0.05	3.9 <sup>b</sup> ±0.13	4.7 <sup>a</sup> ±0.21	4.1 <sup>b</sup> ±0.18	**
Albumin(A)(g/dl)	1.1 <sup>c</sup> ±0.04	1.86 <sup>a</sup> ±0.05	1.6 <sup>b</sup> ±0.05	1.7 <sup>ab</sup> ±0.04	**
Globulin(G)(g/dl)	1.7 <sup>c</sup> ±0.06	2.1 <sup>bc</sup> ±0.14	3.0 <sup>a</sup> ±0.18	2.3 <sup>b</sup> ±0.18	**
A/G ratio	0.45 <sup>b</sup> ±0.03	0.93 <sup>a</sup> ±0.08	0.54 <sup>b</sup> ±0.02	0.78 <sup>a</sup> ±0.06	**
Triglycerides(mg/dl)	101.9 <sup>c</sup> ±2.2	100.1 <sup>c</sup> ±2.0	123.1 <sup>a</sup> ±3.3	114.0 <sup>b</sup> ±1.8	**
Cholesterol(mg/dl)	131.7 <sup>b</sup> ±5.9	133.5 <sup>b</sup> ±5.5	149.5 <sup>ab</sup> ±7.9	155.1 <sup>a</sup> ±6.6	**
Ca (mg/dl)	8.1±0.13	8.3±0.11	8.1±0.12	8.3±0.17	NS
P (mg/dl)	4.6 <sup>c</sup> ±0.19	4.6 <sup>bc</sup> ±0.29	5.4 <sup>a</sup> ±0.15	5.3 <sup>ab</sup> ±0.20	**

<sup>a, b, c</sup> means with the same superscripts within the same row are not significant

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.01)

**Table (6):** Effect of *in ovo* injection with vitamin C on small intestine histology ( $\bar{X} \pm SE$ )

Parameters	Treatments				Sig.
	Control	Saline	2.5%vit.C	5%vit.C	
<b>Duodenum</b>					
villi height(V)( $\mu$ m)	1374.0 <sup>b</sup> ±64.5	1714.2 <sup>a</sup> ±55.9	1241.6 <sup>b</sup> ±42.7	1836.3 <sup>a</sup> ±139.0	**
villi width( $\mu$ m)	121.8 <sup>b</sup> ±17.2	98.5 <sup>b</sup> ±11.5	113.0 <sup>b</sup> ±3.8	160.6 <sup>a</sup> ±9.1	**
Crypt depth(C)( $\mu$ m)	479.1 <sup>b</sup> ±25.7	686.0 <sup>a</sup> ±15.4	621.3 <sup>a</sup> ±42.5	516.1 <sup>b</sup> ±12.9	**
V/C	2.9 <sup>b</sup> ±0.26	2.5 <sup>cb</sup> ±0.07	2.0 <sup>c</sup> ±0.16	3.5 <sup>a</sup> ±0.25	**
<b>Jejunum</b>					
villi height(V)( $\mu$ m)	1188.5 <sup>c</sup> ±36.5	1348.6 <sup>b</sup> ±34.6	1546.5 <sup>a</sup> ±44.2	1468.7 <sup>a</sup> ±28.2	**
villi width( $\mu$ m)	93.7 <sup>b</sup> ±4.8	140.7 <sup>a</sup> ±15.8	175.7 <sup>a</sup> ±13.4	168.7 <sup>a</sup> ±12.1	**
Crypt depth(C) ( $\mu$ m)	402.2±21.6	413.8±47.7	348.8±30.1	413.7±10.7	NS
V/C	3.0 <sup>b</sup> ±0.24	3.5 <sup>b</sup> ±0.37	4.6 <sup>a</sup> ±0.45	3.5 <sup>b</sup> ±0.11	**
<b>Ileum</b>					
villi height(V)( $\mu$ m)	1301.5 <sup>a</sup> ±39.7	1339.5 <sup>a</sup> ±51.3	1332.3 <sup>a</sup> ±32.5	1151.7 <sup>b</sup> ±28.2	**
villi width( $\mu$ m)	138.4±12.4	119.4±12.2	140.3±6.9	151.0±15.6	NS
Crypt depth(C) ( $\mu$ m)	327.6 <sup>a</sup> ±9.8	407.6 <sup>a</sup> ±25.8	221.1 <sup>b</sup> ±9.7	396.7 <sup>a</sup> ±47.5	**
V/C	4.0 <sup>b</sup> ±0.2	3.4 <sup>b</sup> ±0.3	6.1 <sup>a</sup> ±0.3	3.4 <sup>b</sup> ±0.6	**

<sup>a, b, c</sup> means with the same superscripts within the same row are not significant

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.01)

**Table (7) :** Effect of *in ovo* injection with glucose on chick's performance (g/b)

Parameters	Treatments				Sig.
	Control	Saline	5% glucose	10%glucose	
BW <sub>0</sub> <sup>3</sup>	31.5 <sup>b</sup> ±0.4	32.4 <sup>ab</sup> ±0.3	33.4 <sup>a</sup> ±0.5	32.1 <sup>b</sup> ±0.4	*
BW <sup>F</sup> 10 wks	568.4 <sup>b2</sup> ±11.5	552.6 <sup>b</sup> ±10.6	580.3 <sup>b</sup> ±11.1	613.0 <sup>a</sup> ±10.7	**
BWG 0-10wks	536.7 <sup>b</sup> ±11.4	520.3 <sup>b</sup> ±10.5	546.9 <sup>b</sup> ±11.1	580.7 <sup>a</sup> ±10.7	**
FC 0-10wks	2047.3 <sup>a</sup> ±10.9	1840.2 <sup>b</sup> ±14.8	1814.2 <sup>c</sup> ±6.2	1864.3 <sup>b</sup> ±13.9	**
FCR 0-10 wks	3.9 <sup>a</sup> ±0.07	3.6 <sup>b</sup> ±0.07	3.4 <sup>c</sup> ±0.07	3.2 <sup>c</sup> ±0.06	**
Mortality%	7.9	6.0	6.0	6.0	
Hatchability	49.4	65.6	64.7	59.6	

<sup>1</sup>Total number of chicks control =38, saline=50, 5% glucose= 50, 10% glucose=50

<sup>2 a, b, c</sup> means with the same superscripts within the same row are not significant

<sup>3</sup> BW<sub>0</sub> = body weight at day – old, BW<sup>F</sup>= body weight at 10 week- old

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.01)

## In Ovo feeding, vitamin C, glucose, hatchability, performance, gut histology

**Table (8):** Effect of in ovo injection with glucose on carcass traits ( $\bar{X} \pm SE$ )

parameters	Treatments				Sig.
	Control	Saline	5% glucose	10%glucose	
Live weight (g)	550.1±20.4	562.1±25.8	612.3±16.9	603.1±21.3	NS
Dressed carcass(g)	344.8±16.2	341.0±17.1	383.8±10.2	369.0±14.6	NS
Dressed Carcass%	62.5±0.38	60.6±0.50	62.7±0.51	61.1±0.26	NS
Liver weight (g)	13.7±0.50	14.9±0.85	15.1±1.1	15.7±0.80	NS
Liver %	2.5±0.25	2.6±0.26	2.4±0.28	2.61±0.18	NS
Gizzard weight (g)	16.0±0.86	16.6±1.1	18.1±0.59	16.7±1.3	NS
Gizzard%	2.9±0.38	2.9±0.34	2.9±0.25	2.76±0.28	NS
Spleen weight(g)	1.7±0.11	2.0±0.20	2.1±0.13	1.7±0.20	NS
Spleen%	0.31±0.10	0.36±0.15	0.3±0.13	0.28±0.12	NS
Bursa weight(g)	1.4±0.28	1.6±0.30	1.7±0.30	1.5±0.14	NS
Bursa%	0.26±0.29	0.29±0.25	0.28±0.26	0.25±0.11	NS

NS = not-significant

**Table (9):** Effect of *in ovo* injection with glucose on weight and length of digestive organ ( $\bar{X} \pm SE$ )

Parameters	Treatments				Sig.
	Control	Saline	5% glucose	10%glucose	
Esophagus length(cm)	16.0±0.75	17.2±0.68	16.0±1.01	17.1±0.47	NS
Duodenum length(cm)	22.0 <sup>b</sup> ±0.54	22.5 <sup>b</sup> ±0.85	22.0 <sup>b</sup> ±0.97	26.0 <sup>a</sup> ±1.9	*
Duodenum weight (g)	6.4±0.64	7.3±0.68	6.4±0.79	7.6±0.37	NS
Jejunum length (cm)	49.9±2.4	52.1±3.2	48.6±1.9	46.3±2.1	NS
Jejunum weight (g)	9.3±0.63	11.3±0.84	11.0±1.2	10.8±0.67	NS
Ileum length(cm)	51.9±1.3	52.5±3.1	50.5±2.9	52.8±2.2	NS
Ileum weight (g)	6.5 <sup>b</sup> ±0.26	7.7 <sup>ab</sup> ±0.50	7.2 <sup>ab</sup> ±0.43	8.2 <sup>a</sup> ±0.65	*
Cecum length(cm)	23.5±0.76	23.0±0.77	24.8±0.70	24.5±1.1	NS
Rectum length(cm)	6.9±0.39	7.1±0.43	7.4±0.35	6.6±0.62	NS

<sup>a, b, c</sup> means with the same superscripts within the same row are not significant

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.0)

**Table (10):** Effect of *in ovo* injection with glucose on blood parameters ( $\bar{X} \pm SE$ )

Parameters	Treatments				Sig.
	Control	Saline	5% glucose	10%glucose	
Glucose(mg/dl)	149.7±4.4	158.8±5.1	147.0±4.5	155.2±7.5	NS
Protein(g/dl)	2.9 <sup>b</sup> ±0.05	3.9 <sup>a</sup> ±0.13	4.3 <sup>a</sup> ±0.25	4.2 <sup>a</sup> ±0.25	**
Albumin(A)(g/dl)	1.1 <sup>b</sup> ±0.04	1.86 <sup>a</sup> ±0.05	1.8 <sup>a</sup> ±0.11	1.7 <sup>a</sup> ±0.08	**
Globulin(G)(g/dl)	1.7 <sup>b</sup> ±0.06	2.1 <sup>ab</sup> ±0.14	2.4 <sup>a</sup> ±0.20	2.4 <sup>a</sup> ±0.26	**
A/G ratio	0.45 <sup>b</sup> ±0.03	0.93 <sup>a</sup> ±0.08	0.77 <sup>a</sup> ±0.07	0.79 <sup>a</sup> ±0.12	**
Triglycerides(mg/dl)	101.9 <sup>c</sup> ±2.2	100.1 <sup>c</sup> ±2.0	119.5 <sup>b</sup> ±4.1	134.0 <sup>a</sup> ±3.9	**
Cholesterol(mg/dl)	131.7 <sup>b</sup> ±5.9	133.5 <sup>b</sup> ±5.5	149.9 <sup>a</sup> ±2.7	152.2 <sup>a</sup> ±6.9	**
Ca (mg/dl)	8.1±0.13	8.3±0.11	8.0±0.16	8.3±0.12	NS
P (mg/dl)	4.6 <sup>b</sup> ±0.19	4.6 <sup>b</sup> ±0.29	5.5 <sup>a</sup> ±0.09	4.8 <sup>b</sup> ±0.23	**

<sup>a, b, c</sup> means with the same superscripts within the same row are not significant

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.01)

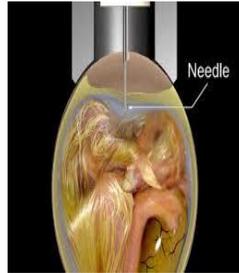
**Table (11):**Effect of *in ovo* injection with glucose on small intestine histology ( $\bar{X} \pm SE$ )

Parameters	Treatments				Sig.
	Control	Saline	5% glucose	10%glucose	
<b>Duodenum</b>					
villi height(V) (µm)	1374.0±64.5 <sup>c</sup>	1714.2±55.9 <sup>ab</sup>	1444.0 <sup>bc</sup> ±70.6	1764.5 <sup>a</sup> ±194.6	**
villi width (µm)	121.8 <sup>b</sup> ±17.2	98.5 <sup>b</sup> ±11.5	196.8 <sup>a</sup> ±28.2	190.8 <sup>a</sup> ±15.1	**
Crypt depth(C)	479.1 <sup>c</sup> ±25.7	686.0 <sup>a</sup> ±15.4	577.6 <sup>b</sup> ±38.2	565.8 <sup>bc</sup> ±66.9	**
V/C	2.9 <sup>ab</sup> ±0.26	2.5 <sup>b</sup> ±0.07	2.5 <sup>b</sup> ±0.12	3.1 <sup>a</sup> ±0.22	*
<b>Jejunum</b>					
villi height(V) (µm)	1188.5 <sup>c</sup> ±36.5	1348.6 <sup>b</sup> ±34.6	1513.3 <sup>a</sup> ±27.4	1428.0 <sup>ab</sup> ±76.1	**
villi width (µm)	93.7 <sup>b</sup> ±4.8	140.7 <sup>a</sup> ±15.8	149.1 <sup>a</sup> ±14.7	141.0 <sup>a</sup> ±12.9	**
Crypt depth(C), µm	402.2±21.6	413.8±47.7	480.0±17.3	459.2±47.6	NS
V/C	3.0±0.24	3.5±0.37	3.1±0.08	3.4±0.48	NS
<b>Ileum</b>					
villi height(V) (µm)	1301.5 <sup>a</sup> ±39.7	1339.5 <sup>a</sup> ±51.3	1142.2 <sup>b</sup> ±27.1	1166.3 <sup>b</sup> ±30.3	**
villi width (µm)	138.4±12.4	119.4±12.2	136.0±6.3	126.0±14.3	NS
Crypt depth(C), µm	327.6 <sup>b</sup> ±9.8	407.6 <sup>a</sup> ±25.8	350.5 <sup>ab</sup> ±11.6	337.6 <sup>b</sup> ±33.0	*
V/C	4.0±0.16	3.4±0.31	3.2±0.12	3.7±0.33	NS

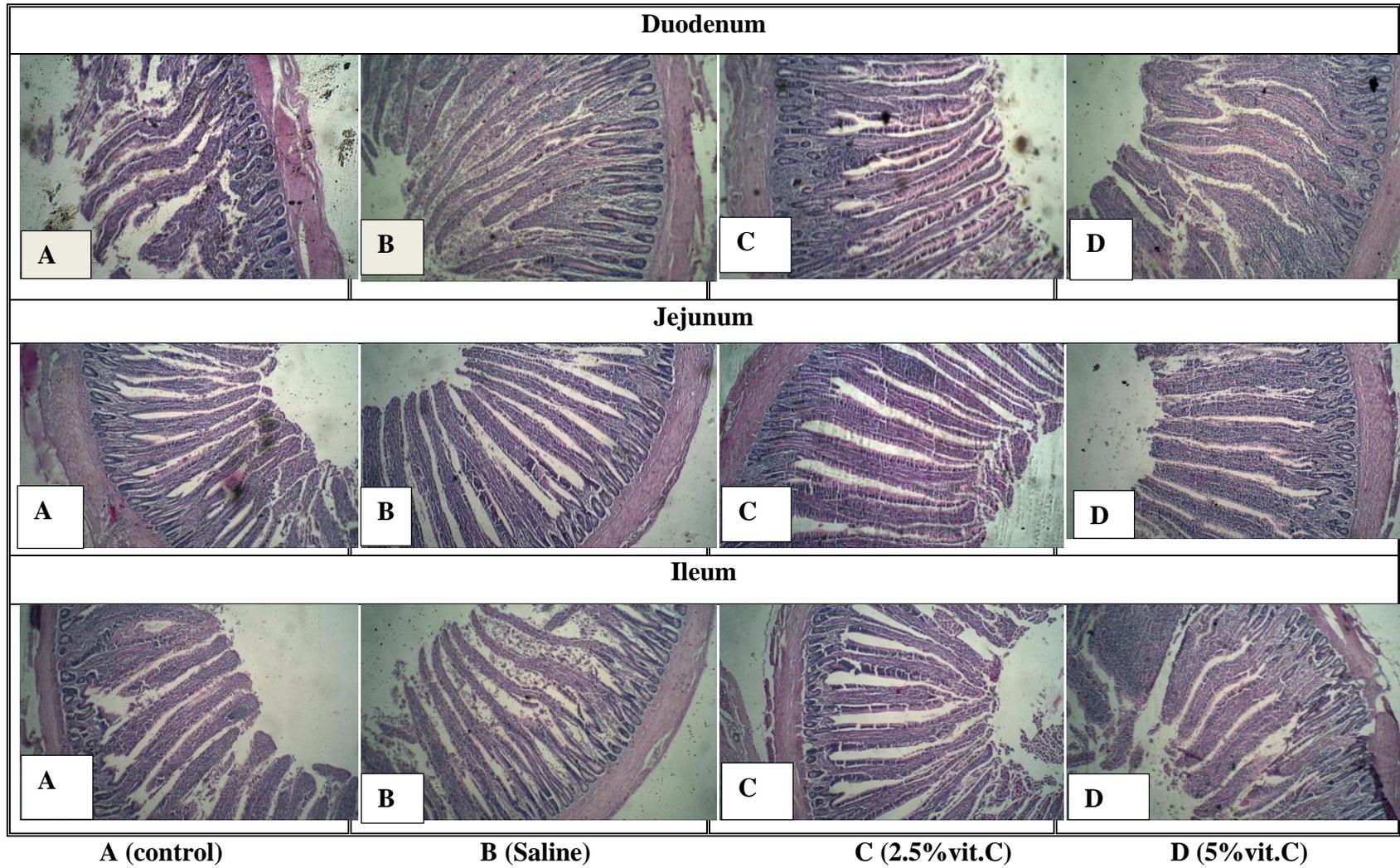
<sup>a, b, c</sup> means with the same superscripts within the same row are not significant

NS = not-significant \* Significant (P<0.05) \*\* Significant (P<0.01)

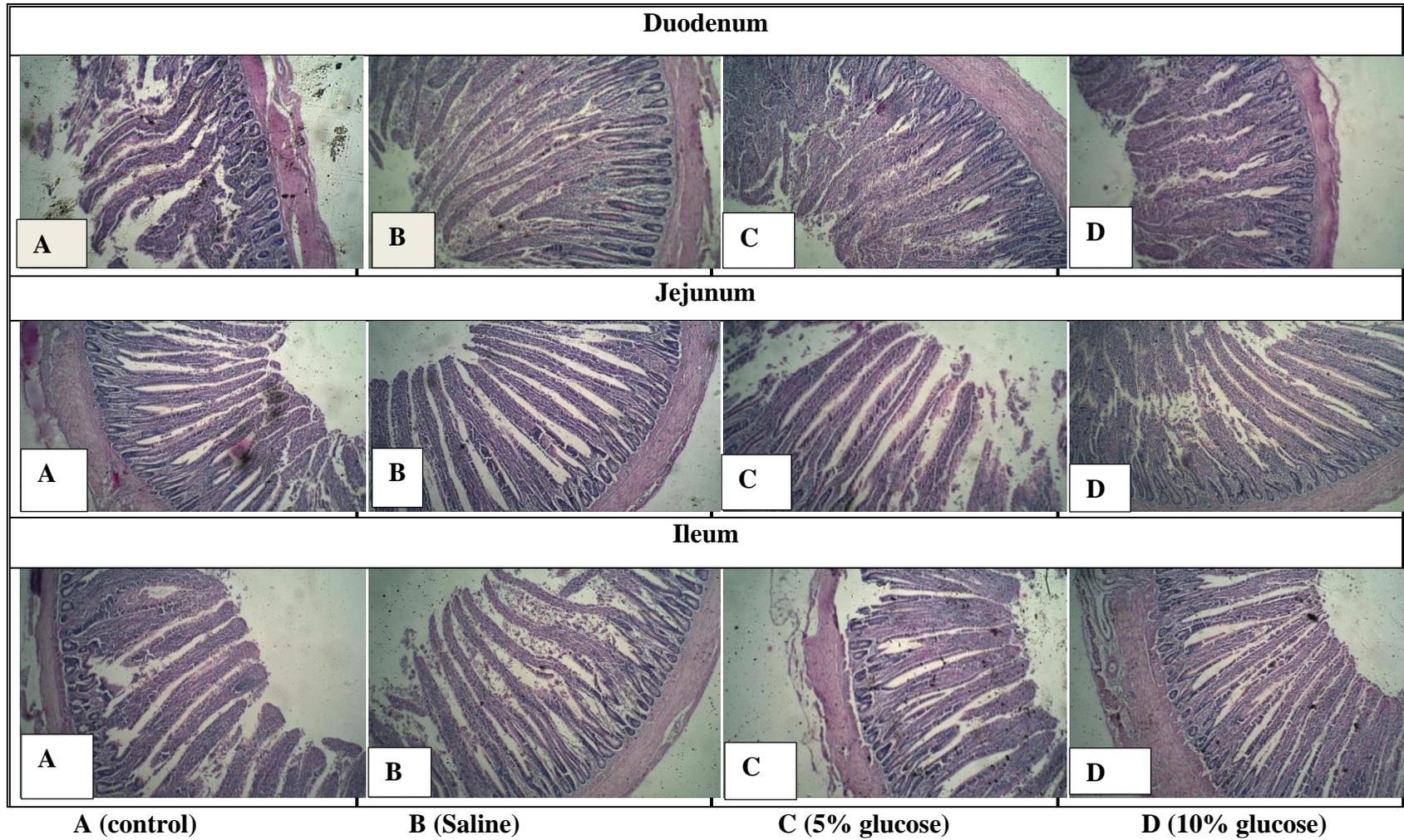
**In Ovo feeding, vitamin C, glucose, hatchability, performance, gut histology**



**Figure (1):** In Ovo injection (Source: Sharma, J and B. Burmester (1982))



**Figure (2):** Microscope photo of intestinal villi from duodenum, jejunum and ileum in different treatments at in ovo injection with vitamin C (Maj, 4x



**A (control)**                      **B (Saline)**                      **C (5% glucose)**                      **D (10% glucose)**  
**Figure (3):** Microscope photo of intestinal villi from duodenum, jejunum and ileum in different groups at in ovo injection with glucose (Maj, 4x10)

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### **In Ovo feeding, vitamin C, glucose, hatchability, performance, gut histology**

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تأثير التغذية بحقن البيض بفيتامين "ج" أو الجلوكوز علي الأداء الانتاجي لكتاكتيت الدندراوي بعد الفقس

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اجريت الدراسة بهدف تقييم تأثير التغذية بالحقن اثناء الحياة الجنينية بفيتامين "ج" أو الجلوكوز علي نسبة الفقس ، و الاداء الانتاجي بعد الفقس ، و صفات الذبيحة ، و هستولوجيا القناة الهضمية. تم استخدام مجموعتين من بيض الدندراوي المخصب (400 بيضة لكل مجموعة في تجربتين منفصلتين. تم فحص بيض المجموعتين في اليوم ال14 من التفريخ لاستبعاد الاجنة غير الحية. و تم تقسيم بيض التجربة الأولى بالتساوي الي اربع معاملات (100 بيضة /معاملة) و تم حقن البيض في الامنيون في اليوم ال18 كالتالي: المعاملة الاولى (كنترول سلبي بدون حقن)، المعاملة الثانية (كنترول موجب حقن 0.1 ملي محلول ملحي 0.9جم/لتر)، المعاملة الثالثة (حقن 0.1 ملي محلول 2.5% فيتامين "ج" ذائب في الماء) ، المعاملة الرابعة (حقن 0.1 ملي محلول 5% فيتامين "ج" ذائب في الماء). تم معاملة البيض في التجربة الثانية بنفس طريقة التجربة الاولى و تم الحقن في اليوم ال18 في الامنيون كالتالي: المعاملة الاولى (كنترول سلبي بدون حقن)، المعاملة الثانية (كنترول موجب حقن 0.1 ملي محلول ملحي 0.9جم/لتر)، المعاملة الثالثة (حقن 0.1 ملي محلول 5% جلوكوز ذائب في الماء) ، المعاملة الرابعة (حقن 0.1 ملي محلول 10% جلوكوز ذائب في الماء). تم تقسيم الكتاكتيت الفاقسة من كل تجربة الي 3 مكررات / معاملة و تربيتهم حتي عمر 10 اسابيع. اظهرت كتاكتيت المعاملة الرابعة (تجربة 1) الفاقسة من بيض محقون ب5% فيتامين "ج" تحسنا ( $P < .01$ ) في الاداء في وزن الجسم، و الزيادة في وزن الجسم، و معدل التحويل الغذائي و سجلت اقل نسبة نفوق. غير ان قياسات الذبيحة لم تظهر اي اختلافات معنوية بين المعاملات. علاوة علي ذلك كانت قياسات الدم جميعها ضمن القيم الفسيولوجية الطبيعية. كما اظهرت الفحوصات الهستولوجية للقناة الهضمية تأثير ايجابي ( $P < .01$ ) لمستوي 5% فيتامين "ج" علي ارتفاع و عرض الخملات للاثني عشر. اظهرت نتائج التجربة الثانية تحسن ( $P < .01$ ) معنوي لاداء الكتاكتيت الفاقسة من بيض محقون ب 10% جلوكوز . كما سجل الفحص الهستولوجي لنفس المجموعة اعلي ( $P < .01$ ) قيم لطول و عرض الخملات للاثني عشر. و يستنتج من ذلك ان حقن بيض الدندراوي عند اليوم 18 من التفريخ بمقدار 0.1 ملي 5% فيتامين "ج" او 0.1 ملي 10% جلوكوز أدى الي تحسن في اداء الكتاكتيت بعد الفقس و في الصفات الهستولوجية للقناة الهضمية المتعلقة بامتصاص الغذاء.