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Impact of injecting eggs with Ascorbic acid and Glucose on lengths of Digestive Tract parts of Japanese Quail

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I. Abstract

This study was conducted to demonstrate the effect of injecting Japanese quail eggs with ascorbic acid and glucose and their mixture on the length of the parts of the digestive tract. Six hundred fertilized eggs were purchased from one of the private hatcheries in Baghdad/Iraq. Distribute the eggs into four equal groups (150 eggs for each), then injected with nutrient solutions as follows: First group (T1): without injection (control), the second group (T2): 0.1 ml ascorbic acid, 1% concentration, the third group (T3): 0.1 ml glucose, 1% concentration, and the fourth group (T4): A mixture of (0.1 ml ascorbic acid + 0.1 ml of glucose). Each group consists of 3 replicates (50 eggs) per replicate. After hatching, the birds were fed two diets, starter and final, for 6 weeks. The results of the study showed a significant increase ($P \geq 0.05$) in most of the lengths of the parts of the digestive tract. This indicates a positive indicator of improvement and increase in absorption in the digestive tract and the development of its ability to perform.

Keywords: *Ascorbic acid, Glucose, Injection, Egg, Quail*

II. Introduction

Injecting hatching eggs with vitamins such as ascorbic acid & nutrients such as glucose is of great importance & benefit in nourishing embryo inside egg, results of which are reflected positively in period immediately following hatching. Therefore, the hatched birds will have an improvement in enzymatic activity & activity & growth of villi in digestive tract, which increases ability to digest & absorb, which is reflected in health of hatched birds & improves their productive performance as a result of rapid development of their gastrointestinal system. (Kattan and Shahid, 2021). Ascorbic acid, or what is called vitamin C, is a water-soluble vitamin that acts as an antioxidant & anti-stress, & also acts as an immune regulator in birds. (Zhu et al., 2019). Ascorbic acid reduces corticosterone, acting as an anti-stress agent. (Ferronato et al., 2024). Due to its high nutrient content, ascorbic acid can promote glucose production, which helps adapt embryos during incubation period (Zhu et al., 2019). During incubation, especially on third or fourth day, embryo works to manufacture ascorbic acid due to its lack in hatching eggs. (Mousstaaid et al., 2022), on fifteenth day concentration begins Ascorbic acid decreases (Wilson & Jaworski, 1992). Scientific research & recent studies have confirmed that it is possible to inject eggs with nutrients & vitamins during incubation period due to effect of injections on improving & increasing egg hatching rate, as well as ensuring safety of embryos & enhancing their growth. (Tollett, 1990; Zakaria et al., 1998). Vitamins are injected into the hatching eggs as an important protection & to maintain activity of embryos inside egg before hatching process, as well as birds in period following hatching process (Surai, 2000). For this reason, injecting ascorbic acid into hatching eggs helps reduce soft damage from exposure of embryos to stress as a result of high temperature (Ş ahin & Küçük, 2001). Glucose is

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considered the most important source of energy for all living organisms (Strier, 1995), During stages of its development & formation, fetus is in dire need of nutrients & solutions that are considered an energy source that enhances growth of fetus inside egg (Palmer and Gillette, 1991; Vieira, 2007). Therefore, glucose must be injected into hatching eggs because glucose is only one that is absorbed during first day of hatching during digestion & absorption process, after which its absorption decreases on fourth day (Noy & Sklan, 2001).

III. Materials & Methods

Hatching eggs

Six hundred fertile quails eggs (Coturnix Japonica) with average egg-weight of 15g -17g & with same age were purchased from general authority for agricultural research / abu ghraib, Baghdad. In this farm, birds were kept & grown up under the standard condition of breeding.

Egg injection method

Before performing egg injection procedure, clean wide end of egg with 70% ethyl alcohol. After that, egg shell is pierced with a needle (capacity 1 ml), & this is done very carefully to ensure that outer membrane of egg is not scratched. Glucose & ascorbic acid solutions were injected into egg albumen area (Akhlaqi et al., 2013). The holes at injection site are then closed with melted paraffin wax. eggs were numbered & kept for 3 days until end of incubation period.

Preparing nutrient solutions

1. (1 g) of ascorbic acid was added to a volumetric flask gradually, after which volume was increased to (100 ml) with sterile distilled water until its concentration became 1%.

2. (1 g) of Glucose was added to a volumetric flask gradually, after which volume was increased to (100 ml) with sterile distilled water until its concentration became 1%.

3. For mix (0.50 g of ascorbic acid & 0.50 g of Glucose) was added to a volumetric flask gradually, after which volume was increased to (100 ml) with sterile distilled water until its concentration became 1%.(Al-Hassani & Al-Asadi,2009).

Traits Measurement

Birds in 6 week old, 30 birds from each group were randomly weighed (10 for each replicate). After that, birds were slaughtered, dissected, & organs of digestive tract were extracted, starting from esophagus to end of rectum. organs were measured using a special measuring ruler called a vernier, unit of measurement. In millimeters for parts of digestive tract, which are total digestive tract. small intestine includes: Duodenum, Jejunum, Illum & large intestine includes ceca & colon. Total small intestine.

Statistical analysis:

Using Complete Randomiz Design (CRD), data were analyzed, & using Duncan multinomial test (1955), differences between means were compared & ready-made statistical program (SAS, 2001) was used.

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Results

Duodenum length

Table (1) shows that injection groups led to significant differences in duodenal length between groups, as the glucose + ascorbic acid group (mixed) was significantly superior (P<0.05),indicating a synergistic effect of both supplements. while lowest significant difference was recorded in control group.

Table (1) impact of injecting ascorbic acid, glucose & their mixture into Duodenum length of quail birds (mean ± standard error)

Treated group	Length (cm)	Length / Body length (%)	Relative weight (g/100g BW)
Control	11.38 ± 0.80[a]	7.40 ± 0.52[a]	0.39 ± 0.01[a]
Glucose	12.80 ± 0.86[b]	8.29 ± 0.59[b]	0.45 ± 0.03[b]
Ascorbic Acid	13.49 ± 0.77[c]	8.67 ± 0.54[c]	0.49 ± 0.02[c]
Mixed	**14.60 ± 0.93[d]**	**9.19 ± 0.65[d]**	**0.54 ± 0.03**

*Different letters vertically mean there are significant differences at the significance level (0.05)

Jejunum length

Table (2) shows that injection groups led to significant differences in Jejunum length between groups, as the glucose + ascorbic acid group (mixed) was significantly superior (P<0.05),indicating a synergistic effect of both supplements. while lowest significant difference was recorded in control group.

Table (2) impact of injecting ascorbic acid, glucose & their mixture into eggs on lengths of Jejunum parameters of quail birds (mean ± standard error.

Treated group	Length (cm)	Length / Body length (%)	Relative weight (g/100g BW)
Control	23.10 ± 1.19[a]	15.29 ± 0.82[a]	0.88 ± 0.04[a]
Glucose	25.72 ± 1.32[b]	17.05 ± 0.94[b]	1.00 ± 0.06[b]
Ascorbic Acid	26.90 ± 1.27[c]	17.61 ± 0.89[c]	1.07 ± 0.07[c]
Mixed	**29.22 ± 1.39[d]**	**18.70 ± 1.00[d]**	**1.19 ± 0.08[d]**

*Different letters vertically mean there are significant differences at the significance level (0.05)

Ileum length

Table (3), Note that impact of injection groups led to significant differences (P<0.05) in the glucose + ascorbic acid group (mixed), as it was significantly superior to other groups and control group. in length of ileum.

Table (3) impact of injecting ascorbic acid, glucose & their mixture into eggs on lengths of Ileum length of quail birds (mean ± standard error

Treated group	Length (cm)	Length / Body length (%)	Relative weight (g/100g BW)
Control	17.85 ± 1.00[a]	11.74 ± 0.67[a]	0.70 ± 0.03[a]
Glucose	19.84 ± 1.15[b]	12.80 ± 0.77[b]	0.80 ± 0.05[b]
Ascorbic Acid	20.72 ± 1.07[c]	13.25 ± 0.74[c]	0.85 ± 0.05[c]
Mixed	**22.06 ± 1.24[d]**	**14.01 ± 0.82[d]**	**0.92 ± 0.06[d]**

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*Different letters vertically mean there are significant differences at the significance level (0.05)

Ceca length

Table (4) showed that injection of glucose or ascorbic acid alone resulted in moderate increases, whereas the combined treatment produced the highest cecal lengths ($P < 0.05$), as it was significantly superior to other groups and control group.

Table (4) impact of injecting ascorbic acid, glucose & their mixture into eggs on Ceca length of quail birds (mean ± standard error).

Treated group	Length (cm)	Length / Body length (%)	Relative weight (g/100g BW)
Control	8.39 ± 0.49^{a}	5.67 ± 0.30^{a}	0.33 ± 0.00^{a}
Glucose	9.28 ± 0.54^{b}	6.19 ± 0.35^{b}	0.39 ± 0.01^{b}
Ascorbic Acid	9.75 ± 0.52^{c}	6.48 ± 0.33^{c}	0.41 ± 0.01^{c}
Mixed	**10.59 ± 0.60^{d}**	**7.00 ± 0.39^{d}**	**0.46 ± 0.02^{d}**

*Different letters vertically mean there are significant differences at the significance level (0.05)

Colon length

Table (5) noted that injection of glucose or ascorbic acid alone resulted in moderate increases, whereas the combined treatment produced the highest colon lengths ($P < 0.05$), as it was significantly superior to other groups and control group.

Table (5) impact of injecting ascorbic acid, glucose & their mixture into eggs on Colon length of quail birds (mean ± standard error).

Treated group	Length (cm)	Length / Body length (%)
Control	6.32 ± 0.35^{a}	4.27 ± 0.24^{a}
Glucose	6.93 ± 0.41^{b}	4.62 ± 0.29^{b}
Ascorbic Acid	7.29 ± 0.43^{c}	4.85 ± 0.31^{c}
Mixed	**7.86 ± 0.47^{d}**	**5.19 ± 0.33^{d}**

*Different letters vertically mean there are significant differences at the significance level (0.05)

Total small intestine lengths

Table (6), Note that injection of the glucose + ascorbic acid group(mixed) recorded the maximum total small intestine length, reflecting enhanced intestinal growth, whereas the other groups treatment produced the moderate improvement in total small intestine length compared with the control.

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Table (6) impact of injecting ascorbic acid, glucose & their mixture into eggs on Total small intestine parameters of quail birds (mean ± standard error).

Treated group	Length (cm)	Length / Body length (%)	Relative weight (g/100g BW)
Control	53.39 ± 2.26[a]	35.47 ± 1.54[a]	2.00 ± 0.10[a]
Glucose	59.42 ± 2.45[b]	39.18 ± 1.70[b]	2.29 ± 0.13[b]
Ascorbic Acid	61.17 ± 2.34[c]	40.56 ± 1.67[c]	2.45 ± 0.14[c]
Mixed	**66.92 ± 2.69[d]**	**42.94 ± 1.86[d]**	**2.69 ± 0.17[d]**

*Different letters vertically mean there are significant differences at the significance level (0.05)

Total digestive tract length

Table (7) showed, that injection of the glucose + ascorbic acid group(mixed) recorded the maximum total digestive tract length, reflecting enhanced intestinal growth, whereas the other groups treatment produced the moderate improvement in total small intestine length compared with the control.

Table (7) impact of injecting ascorbic acid, glucose & their mixture into eggs on Total digestive tract length of quail birds (mean ± standard error).

Treated group	Length (cm)	Length / Body length (%)
Control	68.13 ± 2.88[a]	45.46 ± 1.95[a]
Glucose	75.66 ± 3.10[b]	49.96 ± 2.13[b]
Ascorbic Acid	79.24 ± 3.03[c]	52.01 ± 2.09[c]
Mixed	**85.42 ± 3.36[d]**	**55.00 ± 2.32[d]**

*Different letters vertically mean there are significant differences at the significance level (0.05)

Discussion

Table (1) showed mixture group was significantly superior to the other groups, followed by glucose & ascorbic acid groups, respectively, while least significant difference was in control group, when ascorbic acid & glucose were injected into quail eggs & their effect on duodenum length was demonstrated. This is also what Uni & Ferket, (2019) found to development of digestive tract parts of birds as a result of injecting nutrient solutions into eggs. Perhaps reason for morphological development of lengths of digestive tract in birds is rapid, immediate feeding of birds after hatching process.(Noy, & Sklan,1999).

Table (2) showed there were significant differences in jejunum length between groups, where it was noted that mixture group was significantly superior ($P<0.05$), while least significant difference was recorded in control group. As for length of ileum, results of table (3) showed length of ileum was significantly higher ($P<0.05$) in mixture group followed by glucose group. While it was lowest in control group. It is possible that this significant superiority can be attributed to the fact that weights of birds after hatching from egg have a relationship & are linked to lengths of parts of digestive tract. It has a relationship with weight of birds after hatching, & this is Yegani & Korver, (2020)found, where they indicated injection of embryos. Broiler eggs on eighteenth day of incubation with carbohydrates led to improvement & development of intestines through an increase in size of villi & capacity of intestines. surface area of villi in intestines of treatments injected with nutrient solutions is larger than it is in intestines of control group birds. Results of table (4) and table (5) indicated there were significant differences ($P<0.05$) between ceca and colon, with highest significant difference in the mixture group

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in lengths of ceca and colon While recording least significant difference in control group. A significant improvement in intestinal length was observed compared to the control group as a result of ascorbic acid injection. This is attributed to the vitamin's active role in collagen synthesis, cell differentiation, and its role in protecting cells from oxidative stress; these factors, in turn, protect intestinal tissue (Khan et al., 2022). In addition to what has been mentioned, ascorbic acid plays a major role in the regeneration of epithelial cells, which may be the reason for the increase in the length of the intestines (Pires et al., 2021). As a result of the synergistic effect between the antioxidant protection by ascorbic acid, which supports the synthesis of structural proteins and the provision of metabolic energy by glucosamine, which leads to rapid cell division, and consequently improved growth of intestinal tissue and an increase in its length (Uni et al., 2021). The study's findings are consistent with recent research confirming the important role of in vivo nutrient supplements in promoting digestive system development in birds. Gonçalves et al. (2020) reported improved intestinal function due to intestinal cell proliferation when egg embryos were injected with nutrient solutions, supporting the excellent results observed in the group treated with the mixture. Early metabolic programming during embryonic development is the reason that could explain the positive improvement in the duodenum, jejunum, and ileum in the nutrient-injected groups. According to Uni and Ferket(2020) ,(The marked increase in intestinal length in birds injected with carbohydrates may be attributed to the fact that glucose plays an important role as an energy source during the embryonic stage and after hatching, and may be the reason for the marked development in intestinal lengths in birds injected with nutritional supplements inside the egg (Foy et al., 2019). The improvement in the length of the cecum and colon may reflect microbes and fermentation in treatments injected with nutrients into the egg, and these gut microbes may improve gut health (Bortuzzi et al., 2022). .

Conclusion

Injecting the egg with glucose and ascorbic acid supplements promotes early digestive system growth and increases the productive performance of quail, because improving digestive system growth during early stages is linked to feeding efficiency and health status.

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