Research Article

The Effects of In-Ovo Dipping of Sugarcane Vinegar on Hatching Weight, Hemato-Biochemical Profile and Subsequent Performance of Newly-Hatched Ducklings during Initial Rearing Phase

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Abstract | This study was designed to determine the effects of in-ovo sugarcane vinegar (SV) solution to Sudani duck's eggs with different concentrations on body weight at hatch, hemato-biochemical changes and subsequent performance of newly-hatched ducklings. A total of 1560 fertile eggs used in the current experiment were obtained from Sudani ducks flocks of 33 weeks of age. The eggs were randomly distributed into of five different SV solution concentrates; the first (1st) group without any treatment and served as a control (C), the 2nd group was dipped into distilled water as a vehicle or positive control (SV1). The 3rd group (SV2), the 4th group (SV3) and the 5th (SV4) group were dipped in 0.05, 0.10 and 0.15% of SV solutions for 3 minutes, respectively, at four-time of eggs dipping: at day 10th (T10), day 17th (T17), day 24th (T24) and day 31st of incubation (T31) totaling twenty groups with three replicates of (26 eggs of each). The results showed that ducklings from eggs dipped at SV had significant (P<0.05) better body weight than ducklings hatched from the control and sham groups. The heterophil to lymphocytes ratio (H/L) was significantly (P≤0.01) lower for Sudani ducklings in SV4- group × T24th than those of the other experimental groups. Furthermore, concentrations of serum total protein and their fractions (albumin and globulin) and all lipid profile were insignificant affects by in-ovo different SV solutions, times of eggs dipping and their interaction. The best values of body weight gain, feed conversion ratio and performance index were recorded in that group dipping eggs at 0.05% SV followed by groups dipping at 0.15 or 0.10 % SV throughout the experimental period. According to the results, it can be concluded that in-ovo dipping in 0.0.5% of SV solution, especially on day 10th of incubation has a positive effect on the subsequent performance of newly-hatched ducklings during initial rearing phase.

Keywords | Blood, Body weight, Dipping, Duckling, Hematology, In-ovo, Sugarcane vinegar

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INTRODUCTION

The overall goals of early feeding (in-ovo technique) of embryos by providing the incubated eggs with nutrients are to support the fetus in the formation of body tissues and mitigate the stress during the hatching process (Peebles, 2018; El-Kholy et al., 2021).

One of the in-ovo feeding methods is the administration of exogenous nutrients into ducks fertile eggs by dipping (Al-Asadi and Ibrahim, 2020; El-Kholy et al., 2022). In-ovo supplementation involves the administration of a solution of natural nutrient compounds to modulate enteric development, to improve the hatchling's nutritional status during the transition from embryonic nutrition and

to transfer duckling into the diet digestive competency. Naturally, the developmental environment of ducklings embryo has limited amount of in-ovo energy and nutrients to support embryonic growth and hatching percentage (El-Kholy et al., 2022). Furthermore, until external feeding is resumed, chick growth is dependent on 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 post-hatch development (Uni et al., 2012; Tag El-Din et al., 2018; El-Kholy et al., 2022). Furthermore, in-ovo delivery of nutrients results in the improved early development of the digestive organs during the last quarter of ducklings embryonic development.

A variety of nutrient supplements can be included in the in-ovo supplementing solution in various concentrations (Ghonim et al., 2009; Al-Asadi and Ibrahim, 2020; El-Kholy et al., 2022).

Sugarcane vinegar (SV) is produced by alcoholic fermentation and ethanoic acid fermentation of sugarcane juice (Zheng et al., 2016). Literature has shown that total organic acids (He et al., 2017) and total polyphenol content (Chen et al., 2015) in sugarcane vinegar are 3.65% and 132.08 µg/mL, respectively. Acetic acid is the most common organic acid found in sugarcane vinegar. Oxalic, tartaric, acetic, and succinic acids are among the numerous organic acids found in vinegar (Chen et al., 2015). Ten major phenolic compounds such as caffeic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, ferulic acid, apigenin, coumarin, kaempferol, luteolin, and vanillin have been detected in sugarcane vinegar-based beverages (He et al., 2017). Previous results showed that vinegar has more antioxidant properties than ascorbic acid and gallic acid (Zheng et al., 2016; Lin et al., 2017). The European Union allowed the use of organic acids in poultry production because these are generally considered a weak acid and safe (Adil et al., 2010). It's a cheaper cost of the organic acid which used successfully in folk medicine as sanitizer, antimicrobial, alleviating the effects of high environmental temperatures and reducing the stress of the chick faces during hatching.

Organic acids are natural products of microbial fermentation of carbohydrate nutrients (Diba et al., 2015). So, in recent years, scientists have confirmed that the use of these organic acids as promoter for the production of poultry rather than antibiotics, which improves growth, production and immunity against diseases when added to feed and drinking water (Ragaa and Korany, 2016; Sheoran and Tewatia, 2017), without leaving negative effects or residues harmful to birds, humans and the environment, It is absorbed by the cells of the digestive system and decompose into water and carbon to be a source of energy for these absorbed cells (Dibner and Buttin, 2002). Acetic acid is one of the most important types of organic acids, called vinegar with a concentration of 4% (Diba et al., 2015), acetic acid (CH_3COOH) is a major product in the biotrophic processes within living cells of which Acetylcoenzyme A is produced to produce energy ATP (Lehninger et al., 2012).

Very limited research is available regarding the effect of SV on the health and performance of ducks during initial phase of rearing. Therefore, the objective of the current research was designed to study the appropriate embryonic age for dipping the fertile Sudani duck eggs during incubation period and the optimum level of SV solution concentrate used to dip the eggs on hatching weight, blood hematological and biochemical profiles and subsequent performance of newly-hatched ducklings during initial rearing phase.

MATERIALS AND METHODS

The current study was conducted at El-Serw Waterfowls Research Station, Animal Production Research Institute, Agricultural Research Center, Damietta, Egypt, in collaboration with Faculty of Agriculture, Damietta University, Egypt.

Ethical approval

This research was carried out in accordance with the Animal Care and Use Committee guidelines of the Damietta University, Damietta, Egypt (Approval number: 03/2018/du.edu). The hatching eggs and the ducklings in the experiment were provided proper care and management without unnecessary discomfort.

Solutions preparation

The sugar cane vinegar 5% concentrate was purchased from a local company, Egypt. It was considered as a stock solution in this experiment. The solutions were freshly prepared using distilled water. 10, 20 and 30 ml from the previous solution were diluted with 990, 980 and 970 ml of distilled water to prepare 0.05, 0.10 and 0.15% of SV solutions, respectively.

Experimental procedures

A total number of 1560 fertile Sudani duck (Egyptian Muscovy; is a native bird of Egypt) eggs were weighted around 64 ±1g and distributed according to randomized block experimental design in a (5×4) factorial arrangement, consisting of negative and positive control groups and three different SV solution concentrates; the 1st group without any treatment and served as a control (C), the 2nd group was dipped into distilled water as a vehicle or positive control

(SV1). The 3rd group (SV2), the 4th group (SV3) and the 5th (SV4) group were dipped in 0.05, 0.10 and 0.15% of SV solutions, respectively, at four time of eggs dipping "embryonic ages" at the day 10th (T10), day 17th (T17), day 24th (T24) and day 31st of incubation (T31) totaling twenty groups with three replicates of (26 eggs of each). The eggs put in perforated plastic baskets and submerged in a larger metal container in which the prepared liquid, which has a temperature of 35°C. Egg dipping time was 3 minutes at a temperature of 35 °C according to Meir and Ar (1984). After applications, eggs were dried at 30°C for 15 minutes.

Egg trays were randomly distributed in 'Econom' incubator system multi-stage at 37.4°C and 62-64% relative humidity. Eggs had been turned every one hours until they transferred to the hatching compartment at the 31st day of incubation. At 31st day of incubation the eggs were transferred to the hatcher which kept at 36.9 °C and 76-78 % relative humidity until the end of hatching period. After the end of incubation, all the hatched ducklings were removed from each hatch basket and counted.

Growth performance parameters

After the quality sorting, a total number of nine hundred unsexed healthy ducklings one day-old produced from them were transported to a private farm, located in Hajaja village, Damietta, Damietta Governorate, Egypt. Live body weight of duckling (LBW, g) was individually weighed to the nearest 0.1g before offering rations at 1st and 21 days of age. It's randomly assigned into completely randomly (5×4) factorial with 3 replicates according to their SV concentrate and EA were penned separately (15 ducklings of each). All ducklings were reared under similar managerial and hygienic conditions. Floor pens covered with wheat chaff litter. The starting brooder temperature was 32°C during the first 3 days, then decreased gradually while light was 24 hours per day at the first two days of housing after that lighting was reduced to constant 21 hours daily throughout the remain period. Feed in mash form and water had offered *ad libitum*. The starter diet (20 % CP and 2850 kcal of ME/ kg) was formulated from plant origin to exceed the National Research Council recommendations (NRC, 1994) as shown in Table 1. At the end of 21th days of ducklings age, all ducklings were individually weighed. Body weight gain (BWG, g) was calculated. Feed consumption (FC, g) and mortality for each replicate were daily recorded. No mortality was recorded during the whole experimental period. Feed conversion ratio (FCR) and performance index (PI) were calculated according to North (1984).

Hematological and serum biochemical parameters

At hatch, blood samples were randomly collected from six ducklings per treatment during slaughter in two separate tubes The 1st heparinized tube was immediately used for hematological parameters; hemoglobin concentration (Hb), red blood cell counts (RBCs, $\times 10^6/mm^3$) and total leukocytes counts (WBCs, $\times 10^3/mm^3$). Total white blood cells were counted by hemocytometer, while heterophils (H, %) and lymphocytes (L, %) were counted in blood smears by using Wright's stain technique, then H: L ratio was calculated.

Table 1: Composition and calculated analysis of the basal diet fed to Sudani ducklings throughout the experimental period.

Ingredients	%
Yellow corn	65.95
Soyabean (44%)	30.25
Wheat bran	0.0
Di-calcium phosphate	1.70
Limestone	1.40
Vit & Min. premix*	0.30
NaCl	0.30
DL.Methionine (97%)	0.10
Total	100
Calculated Analysis **	
Crude protein %	20.00
Metabolizable Energy (ME, Kcal/kg)	2850
Calcium %	1.01
Available phosphorus	0.45

* Each 3kg of premix contains 100 million IUVit A; 2 million IU Vit.D3; 10 g Vit.E; 1 g Vit.K3; 1 g Vit B1; 5 g Vit B2; 10 mg Vit.B12; 1.5 g Vit B6; 30 g Niac; 10 g Panto acid; 1g Folic acid; 50 mg Biotin; 300 g Cho.; 50 g Zinc; 4 g Copper; 0.3 g Iodine; 30 g Iron; 0.1 g Selenium; 60g Manganese; 0.1 g Cobalt; and carrier CaCO3 to 3000 g. **According to NRC (1994).

While another non-heparinized blood tube was centrifuged at 3000 rpm for 15 minutes to obtain blood serum that stored at -20°C till analysis. Serum samples were analyzed by using the commercial kits to determine biochemical blood indicators such as serum total protein (TP, g/dl) and albumin (A, g/dl). However, globulin (G, g/dl) value was obtained by subtracting the values of albumin from the corresponding values of total protein. Also, albumin / globulin (A/ G ratio) values were obtained by dividing the values of albumin on the values of globulins according to Coles (1974). Total cholesterol (TCho, mg/dl), triglyceride (Tri, mg/dl), high density lipoprotein (HDL, mg/dl), low density lipoprotein (LDL, mg/dl) were determined according to the method described by Young (1995). Glucose (Glu, mg/dl) determined according to (Ellefson and Caraway, 1976). Calcium (Calc, mg/dl) was determined by commercial kits.

Statistical analysis

Data obtained were statistically analyzed using two-way analysis of using the General linear Model procedure of

SAS (2012) as following model:

$$Y_{ijk} = \mu + SV_i + Tj + (SV \times T)_{ij} + e_{ijk} \text{ (Starting model)}$$

Where;
Y_{ijk} = observed traits; μ = the overall mean; SV_i = Sugar can vinegar solution concentrate effect (j =1, 2, 3, 4 and 5); T_j= Time of eggs dipping effect (i =1, 2, 3 and 4); $(SV \times T)_{ij}$= Interaction effect between SV solution concentrates and time; E_{ijk}= experimental random error.

The Duncan's new multiple range test was used to assess differences between treatment groups. The mean was used to express all of the findings (±SEM). At P<0.05, the statistical significance was recognized.

RESULTS

Duckling new-hatched weight

Effects of in ovo SV dipping and times of eggs dipping on duckling body weight at hatch are presented in Table 2. As presented in Table 2, in ovo SV dipping and times of eggs dipping had significant effect on duckling body weight at hatch (p<.05). Ducklings from eggs dipped at SV had significant (P<0.05) better body weight than ducklings hatched from the control and sham groups (Table 2). The highest duckling body weight was recorded at T10th and 17th in compared to T24th and 31st.

Table 2: Effect of in-ovo dipping in different concentration of sugarcane vinegar solutions at different times of embryonic ages on newly-hatched duckling weight.

Items[1]	Duckling weight, g
Overall mean	41.75
Sugarcane vinegar solution concentrate effect	
C	40.71[b]
SV1	42.26[a]
SV2	42.04[a]
SV3	41.80[ab]
SV4	41.95[a]
SEM	0.41
Sig.	**
Time of eggs dipping effect	
T10	42.22[a]
T17	43.15[a]
T24	40.82[b]
T31	40.81[b]
SEM	0.37
Sig.	**

[1]C= control, SV1= positive control; SV2= 0.05% SV; SV3= 0.10% SV; SV4= 0.15% SV; T10= day 10th of embryonic age; T17= day 17th of embryonic age; T24= day 24th of embryonic age; T31= day 31st of embryonic age. [a, b and c]Means in the same column and effect bearing different superscripts are significantly different (P≤0.05). SEM= standard error mean; ** = P≤0.01

Hematological parameters

Data of hematological traits are presented in Table 3. Data show that there were significant (P<0.05) differences observed among the experimental groups with regard to Hb and L%. However, there were no significant (P>0.05) differences among the experimental groups for another hematological parameter.

Table 3: Effect of in-ovo dipping in different concentration of sugarcane vinegar solutions at different times of embryonic ages on hematological parameters of ducklings at hatch.

Items[1]	Hematological parameters[2]					
	Hb (g/dl)	RBC (×10⁶/mm³)	WBC (×10³/mm³)	H (%)	L (%)	H/L ratio
Overall mean	12.51	2.84	44.68	25.97	64.34	0.40
Sugarcane vinegar solution concentrate effect						
C	12.35[ab]	2.67	47.86	28.69	62.81[b]	0.46
SV1	11.79[b]	2.58	45.29	26.87	63.58[b]	0.42
SV2	13.13[a]	2.89	44.08	25.48	63.76[ab]	0.40
SV3	12.47[ab]	2.98	43.77	24.38	64.53[ab]	0.38
SV4	12.82[ab]	3.08	42.42	24.46	67.01[a]	0.37
SEM	0.39	0.17	1.72	1.75	1.09	0.02
Sig.	**	NS	NS	NS	**	NS
Time of eggs dipping effect						
T10	12.39	2.58[b]	43.67[ab]	20.09[a]	63.22[b]	0.48[a]
T17	12.40	2.55[b]	47.07[a]	28.47[a]	63.00[b]	0.45[ab]
T24	12.68	3.56[a]	41.40[b]	28.00[b]	62.47[b]	0.46[c]
T31	12.57	2.67[b]	46.59[a]	27.34[a]	68.65[a]	0.40[b]
SEM	0.35	0.16	1.54	1.56	0.98	0.02
Sig.	NS	**	**	**	**	**

[1]C= control, SV1= positive control; SV2= 0.05% SV; SV3= 0.10% SV; SV4= 0.15% SV; T10= day 10th of embryonic age; T17= day 17th of embryonic age; T24= day 24th of embryonic age; T31= day 31st of embryonic age. [2]Hb= hemoglobin; RBC= red blood cells; WBC= while blood cells; H= heterophil; L= lymphocyte. [a, b and c]Means in the same column and effect bearing different superscripts are significantly different (P≤0.05). SEM= standard error mean; NS = non-significant; ** = P≤0.01.

Regrading to times of egg's dipping, Table 3 also shows that there were significant (P<0.05) differences observed among the experimental groups with regard to hematological parameters except Hb. The highest values for RBC, WBC, H (%) and L (%) were detected from ducklings produced from eggs dipped in nutritive solution at 24, 17, 10 and 31 d of embryonic ages, respectively. It is clear that, the lowest H/L ratio was recorded in ducklings produced from eggs dipped in nutritive solution at 31 d of embryonic ages.

Blood constituents

Table 4 shows the data of total protein, albumin, globulin and A/G ratio duo to dipping duck eggs in different SV

concentrations solutions at different times of embryonic ages. It is clear that there were no significant (P>0.05) differences observed among the experimental groups, Numerically, the highest values of TP and albumin were recorded for eggs dipped in 0.15% SV solution (SV4-group) in compared to other groups. The lowest values of A/G ratio was detected in control and SV1 and SV3-groups in compared to other experimental groups.

Table 4: Effect of in ovo dipping in different concentration of sugarcane vinegar solutions at different times of embryonic ages on serum total protein, albumin and globulin and A/G ratio of ducklings at hatch.

Items[1]	Total protein (TP, g/dl)	Albumin (A, g/dl)	Globulin (G, g/dl)	A/G ratio
Overall mean	3.24	1.14	2.20	0.52
Sugarcane vinegar solution concentrate effect				
C	3.22	0.98	2.24	0.45
SV1	3.29	1.03	2.26	0.47
SV2	3.28	1.07	2.22	0.50
SV3	3.17	1.01	2.16	0.48
SV4	3.07	1.63	2.11	0.73
SEM	0.10	0.31	0.14	0.12
Sig.	NS	NS	NS	NS
Time of eggs dipping effect				
T10	3.26	1.09	2.17	0.52
T17	3.27	1.43	2.38	0.58
T24	3.43	1.05	2.37	0.44
T31	2.85	0.99	1.86	0.56
SEM	0.18	0.28	0.13	0.11
Sig.	NS	NS	NS	NS

[1]C=control, SV1= positive control; SV2=0.05% SV; SV3=0.10% SV; SV4=0.15% SV; T10=day 10th of embryonic age; T17= day 17th of embryonic age; T24=day 24th of embryonic age; T31=day 31st of embryonic age. [a, b and c]Means in the same column and effect bearing different superscripts are significantly different (P≤0.05). SEM= standard error mean; NS = non-significant.

Regrading to time of eggs dipping, Table 4 also shows that there were significant (P<0.05) differences observed among the experimental groups with regard to total protein and globulin. However, there were no significant (P>0.05) differences among the experimental groups for albumin and A/G ratio. It is interested to notice that the group which eggs were dipped at 17th d of embryonic age recorded the best values for total protein and group which eggs were dipped and 24th d of embryonic age recorded the less values for A/G ratio being the highest and the lowest, respectively as in compared to other experimental groups.

Lipid profile

As shown in Table 5, the effects of dipping of eggs in different SV solutions, times of eggs dipping and the interaction between them on lipids profile were not significant (P>0.05). The birds belong to SV3-group showed numerically lower total cholesterol, triglyceride and LDL than the birds belong to other experimental groups.

Table 5: Effect of in-ovo dipping in different concentration of sugarcane vinegar solutions at different times of embryonic ages on serum lipid profile of ducklings at hatch.

Items[1]	Total cholesterol (mg/ dl)	Triglycerides (mg/ dl)	High density lipoprotein (mg/ dl)	Low density lipoprotein (mg/ dl)
Overall mean	503.83	134.67	160.62	317.58
Sugarcane vinegar solution concentrate effect				
C	511.67	143.42	157.92	333.17
SV1	523.42	148.25	165.75	328.42
SV2	497.83	147.67	160.25	307.92
SV3	470.67	114.75	158.42	287.75
SV4	515.58	119.25	160.75	330.67
SEM	38.05	14.70	12.94	31.82
Sig.	NS	NS	NS	NS
Time of eggs dipping effect				
T10	518.53	151.07	178.40	309.80
T17	557.73	116.40	177.60	363.80
T24	454.27	116.87	149.60	279.80
T31	484.80	154.33	136.87	316.93
SEM	34.03	13.15	11.58	28.46
Sig.	NS	NS	NS	NS

[1]C= control, SV1= positive control; SV2= 0.05% SV; SV3= 0.10% SV; SV4= 0.15% SV; T10= day 10th of embryonic age; T17= day 17th of embryonic age; T24= day 24th of embryonic age; T31= day 31st of embryonic age. [a, b and c]Means in the same column and effect bearing different superscripts are significantly different (P≤0.05). SEM= standard error mean; NS = non-significant.

Serum glucose and calcium concentrations

As shown in Table 6, the effects dipping of eggs in different SV solutions on serum glucose and calcium concentrations were not significant (P>0.05). Times of eggs dipping during incubation had a significant effect on these parameters (P<0.01). Regarding to time of egg's dipping, the present study shows that significant increase in glucose concentration in T17th and T24th in compared to other experimental times. In the ducklings which hatched from eggs dipped at either T17th or T24th age of embryonic ages recorded the highest concentration of glucose while those hatched from eggs dipped at 10th or 31st recorded the highest values of serum-calcium contrast those hatched from other experimental times.

Post-hatch duckling performance

Results presented in Table 7, showed significant effects of the pre-incubation dipping of Sudani duck eggs in

different SV solutions on post-hatch growth performance of ducklings. It is clearly noticed from the present results that, pre-incubation in-ovo dipping with different SV solutions improved significantly (P≤0.05) LBW, BWG, FCR and PI of Sudani ducklings at different ages from hatch to 21th day of age as compared to control groups. The best values of LBW, BWG, FCR and PI were recorded by the group dipping at 0.05% SV followed by groups dipping at 0.15 or 0.10 % SV throughout the experimental period, (from one up to 21 day of age).

Treatment by time of eggs dipping effects

Concerning of duckling weight was significantly affected by the treatment by time of eggs dipping interaction. The maximum duckling weight was observed (P<0.05) on T24 when duckling weight was 44.00±0.82 in the SV4-group; 43.80±0.82 in the SV3-group; 41.36±0.82 in the SV2-group; 40.88±0.82 in the SV1-group versus 40.50±0.82 in the C-group (P<0.05) (Figure 1). There was no treatment × time interactions for the all hematological parameters except lymphocyte % and H/L ratio; all blood metabolites parameters and serum-glucose levels. However, such interactions were significant for H/L ratio and serum-calcium levels (Figures 2 and 3). Ducklings hatched from SV4, had significantly lower H/L ratio on T24; than other SV × experimental time of egg's dipping.

Table 6: Effect of in-ovo dipping in different concentration of sugarcane vinegar solutions at different times of embryonic ages on serum glucose and calcium of ducklings at hatch.

Items[1]	Glucose (mg/dl)	Calcium (mg/dl)
Overall mean	223.22	10.57
Sugarcane vinegar solution concentrate effect		
C	230.92	10.40
SV1	216.75	10.70
SV2	227.75	10.72
SV3	215.08	10.58
SV4	225.58	10.44
SEM	6.21	0.25
Sig.	NS	NS
Time of eggs dipping effect		
T10	211.13^{b}	11.45^{a}
T17	241.47^{a}	9.98^{b}
T24	226.67^{ab}	9.94^{b}
T31	213.60^{b}	10.91^{a}
SEM	5.56	0.22
Sig.	**	**

[1]C= control, SV1= positive control; SV2= 0.05% SV; SV3= 0.10% SV; SV4= 0.15% SV; T10= day 10th of embryonic age; T17= day 17th of embryonic age; T24= day 24th of embryonic age; T31= day 31st of embryonic age. [a, b and c]Means in the same column and effect bearing different superscripts are significantly different (P≤0.05). SEM= standard error mean; NS = non-significant; ** = P≤0.01

Table 7: Effect of in-ovo dipping in different concentration of sugarcane vinegar solutions at different times of embryonic ages on subsequent growth performance of ducklings during first phase of growing period.

Items[1]	Growth performance parameters[2]					
	LBW, (g) at hatch	LBW, (g) at 21 day of age	BWG, (g)	FC, (g)	FCR (g feed/g gain)	PI(%)
Over all mean	41.75	328.58	286.84	424.13	1.50	22.40
Sugarcane vinegar (SV) solution concentrate effect						
C	40.71^{b}	295.33^{b}	254.63^{b}	396.82^{b}	1.57^{a}	19.00^{b}
SV1	42.26^{a}	341.45^{a}	299.21^{a}	439.59^{a}	1.49^{ab}	23.42^{a}
SV2	42.04^{a}	349.60^{a}	307.56^{a}	431.13^{ab}	1.43^{b}	25.13^{a}
SV3	41.80^{ab}	326.74^{a}	284.96^{a}	426.41^{ab}	1.53^{ab}	22.08^{a}
SV4	41.95^{a}	329.80^{a}	287.86^{a}	426.72^{ab}	1.50^{ab}	22.39^{a}
SEM	0.41	10.48	10.54	12.14	0.04	1.03
Sig.	**	**	**	**	**	**
Time of eggs dipping effect						
T10	42.22^{a}	371.72^{a}	329.52^{a}	471.18^{a}	1.45	26.22
T17	43.15^{a}	343.77^{b}	300.62^{b}	443.16^{ab}	1.50	23.45
T24	40.82^{b}	329.23^{b}	288.42^{b}	435.46^{b}	1.53	21.98
T31	40.81^{b}	269.61^{c}	228.80^{c}	346.72^{c}	1.54	17.97
SEM	0.37	9.37	9.42	10.86	0.03	0.92
Sig.	**	**	**	**	NS	**

[1]C= control, SV1= positive control; SV2= 0.05% SV; SV3= 0.10% SV; SV4= 0.15% SV; T10= day 10th of embryonic age; T17= day 17th of embryonic age; T24= day 24th of embryonic age; T31= day 31st of embryonic age. [2]LBW= live body weight at 21 day of age; BWG= body weight gain; FC= feed consumption; FCR= feed conversion ratio; PI= performance index. [a, b and c] Means in the same column and effect bearing different superscripts are significantly different (P≤0.05). SEM= standard error mean; NS= non-significant; ** = P≤0.01

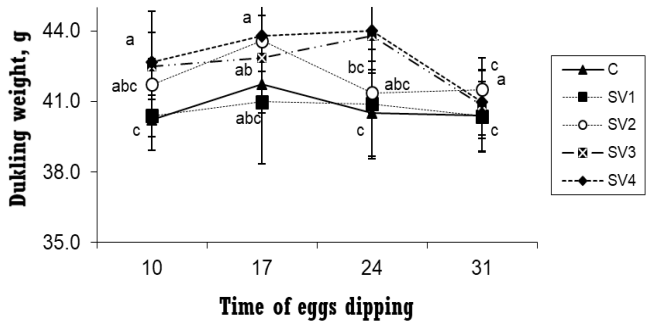

Figure 1: Treatment × time of eggs dipping interaction means (± SEM) for ducklings weight (g). [ab]Means within times with unlike letters differ (P<0.05).

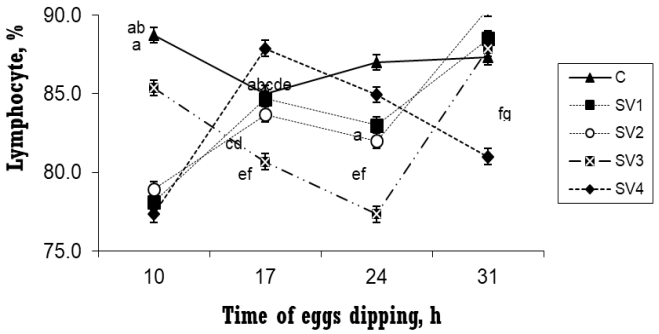

Figure 2: Treatment ×time of eggs dipping interaction means (± SEM) for lymphocyte (%). [ab]Means within times with unlike letters differ (P<0.05).

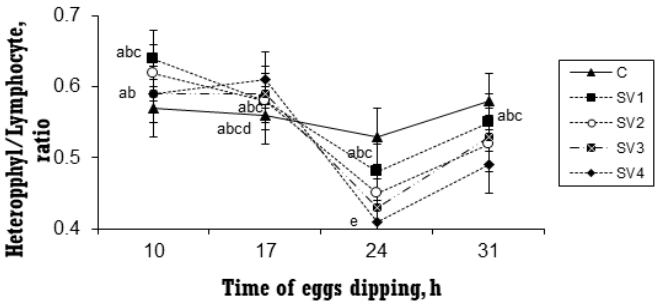

Figure 3: Treatment ×time of eggs dipping interaction means (± SEM) for heterophil/lymphocyte ratio. [ab]Means within times with unlike letters differ (P<0.05).

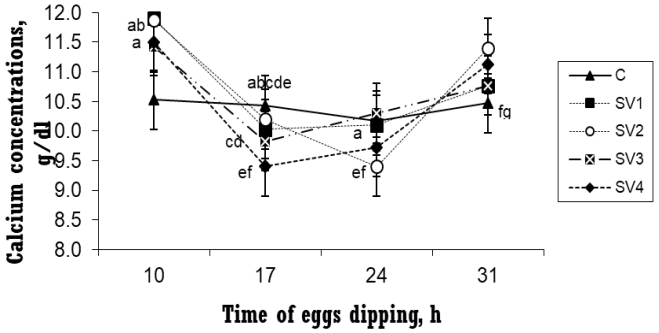

Figure 4: Treatment ×time of eggs dipping interaction means (± SEM) for calcium concentration (mg/dl). [ab]Means within times with unlike letters differ (P<0.05).

Concerning of post-hatch duckling performance all production performance parameters were significantly affected by the treatment by time of eggs dipping interaction. The maximum duckling final body weight at 21 d was observed (P<0.05) on T10 and T17 when duckling final body weight was 441.80±20.96 and 395.42±20.96 in the SV2-group; respectively, versus other experimental groups (Figure 4). Also, such interactions were significant for body weight gain and performance index (Figures 5 and 6). Ducklings hatched from SV2, had significantly higher BWG and PI and significantly (P<0.05) lower FCR on T10 being 400.06±20.9621.07, 43.54±2.06 and 1.29±0.07; respectively; than other SV × experimental time of egg's dipping (Figures 7, 8 and 9).

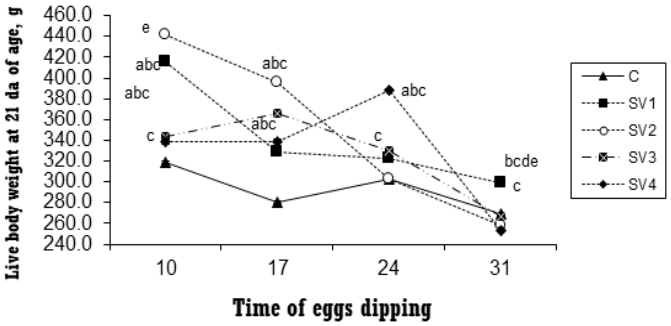

Figure 5: Treatment ×time of eggs dipping interaction means (± SEM) for live body weight at 21 day of age (g). [ab]Means within times with unlike letters differ (P<0.05).

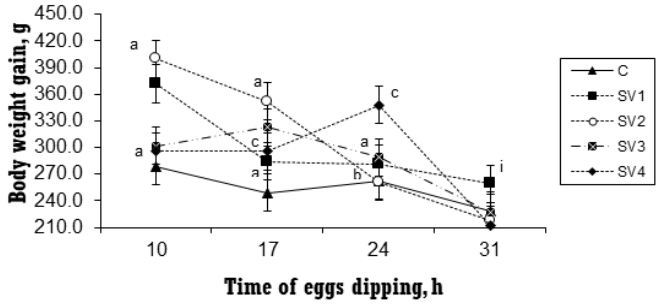

Figure 6: Treatment ×time of eggs dipping interaction means (± SEM) for body weight gain (g). [ab]Means within times with unlike letters differ (P<0.05).

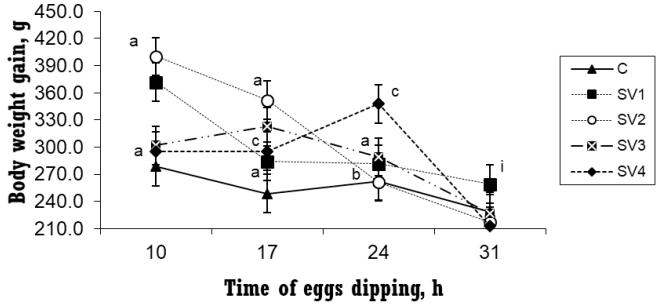

Figure 7: Treatment ×time of eggs dipping interaction means (± SEM) for body weight gain (g). [ab]Means within times with unlike letters differ (P<0.05).

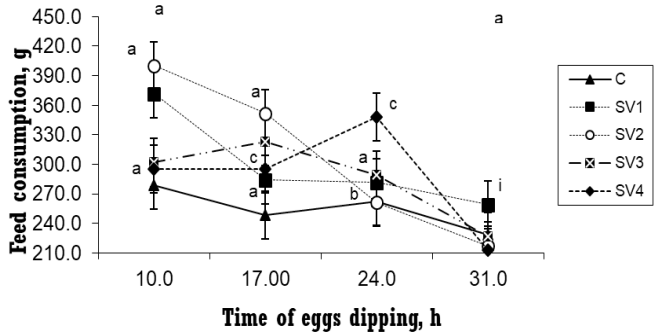

Figure 8: Treatment ×time of eggs dipping interaction means (± SEM) for feed consumption (g). [ab]Means within times with unlike letters differ (P<0.05).

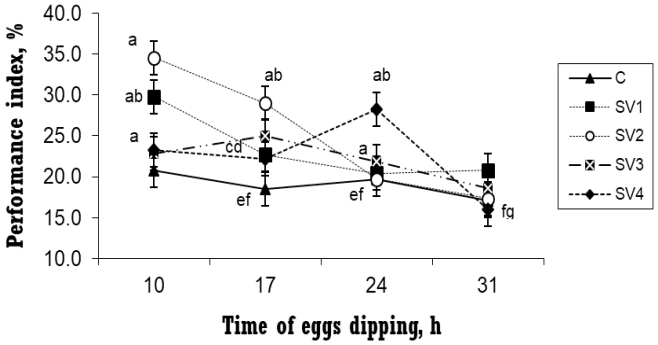

Figure 9: Treatment ×time of eggs dipping interaction means (± SEM) for performance index. [ab]Means within times with unlike letters differ (P<0.05).

DISCUSSION

All reviewed studies relative to in-ovo feeding, including the present study, especially in early embryonic life, were demonstrated that in-ovo accelerated embryo development and improved nutritional status which in turn improved hatching weight and growth rate (Ghonim et al., 2009; El-Din et al., 2018; Al-Asadi and Ibrahim, 2020; El-Kholy et al., 2022).

In the present study, the weight of newly hatched ducklings was significantly higher in groups dipping in SV2 compared to control and SV1 groups especially in either 10th or 17th times of eggs dipping. These results were agreed with Al-Hamed and Al-Eshaki (2019) and El-Kholy et al. (2022). On the other hand, Al-Asadi and Ibrahim (2020) showed that the application of immersion did not affect hatching weight. Previous studies demonstrated that the weight of newly-hatched ducklings is an important predictor of market weight in ducks. In addition, Wilson (1991) indicated that each 1 g of increase in body weight at hatch resulted in 8 to 13 g increase in body weight at market age. According to Samanta et al. (2010), the lower pH of the digesta due to organic acid supplementation may increase pepsin activity and the peptides produced activate the release of hormones such as gastrin and cholecystokinin, resulting in improved digestion and nutrient utilization in birds.

So, the use of organic acids has become more acceptable as in-ovo for ducks. Immersion treatments resulted in an increase weight of ducklings and the ratio of the duckling weight to egg weight, this increase may be due to the SV contain acetic acid (used in the Kreb's Cycle to produce energy ATP) that increase accumulation of glycogen in the liver and muscles before hatching (Kornasio et al., 2011), or to stimulate the evolution and division of satellite cells. These benefits also could be attributed to improved embryo health as a result of therapy with natural white vinegar solution. According to Kirchgessner and Roth (1988), acidification with various weak organic acids such as vinegar improves protein digestibility as well as the digestibility of P, Ca, Mg, and Zn, acts as substrates in the intermediary metabolism, and reduces pathogen colonisation and toxic metabolite production. In addition, Adil et al. (2010) found that dietary addition of organic acids significantly increased the villus height in the duodenum, jejunum, and ileum in the birds fed diets supplemented with organic acids which in turn improved functions of secretion, digestion, and nutrient absorption. Furthermore, they added that treatment with organic acid resulted in a reduction in muscularis thickness, which aids in nutrient digestion and absorption.

Also, where studies indicated a direct relationship between the chick's length and productive performance (Willemsen et al., 2008; Michalczuk et al., 2011). As mentioned by El-Kholy et al. (2022) that dipped eggs in SV led to increase in ducklings length, so this result may be demonstrated the current improved in post-hatch growth performance in SV-groups compared to control group. It is interested to notice that the maximum duckling weight was observed (P<0.05) on T24 when duckling weight was 44.00±0.82 in the SV4-group. This result may be due to metabolic rate at Days 21 and 28 of incubation were the most important predictor variable of this function (Harun et al., 2001).

An increased post-hatch weight of ducklings was the only indication that SV had passed through the shell and was ultimately accessible to the embryo. Presumably, dipping method may cause changes or alterations in the pores, which could influence the shell's gas exchange capabilities. Therefore, accelerated embryo development, by this procedure, improved nutritional status and improved hatching duckling weight and growth rate.

In the present results, the hemoglobin values were altered through in-ovo SV dipping from the all concentrations of SV compared to control and SV1-groups. According to Panda and Cherian (2014), an increase in hemoglobin in SV-groups could indicate that gas perfusion is improving.

Thus, circulating SV may enhance the hematopoietic process by producing a greater number of hemoglobin-rich red blood cells. According to Campbell (1994), an increase in circulating hemoglobin could be also caused by dehydration in the birds, indicating poor either physical quality or management. Hence, it can be stated that blood parameter alteration at the time of hatching occurred due to the presence of SV as an antioxidant. In addition, the increased in hemoglobin values and lymphocyte percentages may have boosted oxygen and nutrient circulation, causing the duckling respiratory rate to increase. Circulating SV may have impacted the hematopoietic process by causing the production of a greater number of hemoglobin-rich red blood cells. In the other hand, in-ovo SV dipping resulted in significant increases in hematopoiesis. These improvements in Hb and Lymphocyte % may enhance blood ability to carrying oxygen to different tissues and in turn improving different metabolic and physiological functions.

The incubator temperature during incubation of duck' eggs was between 37.2 °C and 37.5 °C and the metabolic heat production of the developing embryo is sufficient to raise the internal egg temperature by 1.5 °C to 2 °C (Makram and Abdel-Azim, 2018) that is above the incubator temperature. This may contribute the stress in embryos especially at late period of incubation. So, dipped eggs in SV especially at 31st may be suppress corticosterone synthesis and/ or release from adrenal cortex, which in turn could be play an important role in alleviating stress effects and hence increased the lymphocytes %. These results may ensure that dipped eggs in SV at 31st acted as a good anti-stressors agent. In confirmation of the previous results, in-ovo administration of SV significantly ($P<0.01$) affect Hb, Lymphocyte and H/L ratio. Organic acids were found to have a favorable influence on the humoral immune response by a number of researchers (Kamal and Ragaa, 2014), due to its important role in enhancing the activity of antioxidant enzymes in the blood such as glutathione peroxidase and superoxide dismutase (El-Naggar and Abo El-Maaty, 2017). Also, sugarcane vinegar contains polyphenols and flavonoids compounds that act as strong antioxidants for reducing oxidative stress (Li et al., 2020). The current results are similar to those obtained by El-Naggar and Abo El-Maaty (2017) who found that hemoglobin content was increased in ducks fed a diet supplemented with citric acid. Similarly, El-Kholy et al. (2018 and 2020) reported that hemoglobin content was increased, while H/L ratio was significantly decreased in Domyati ducks.

The heterophil to lymphocytes ratio (H/L) was significantly ($P\leq0.01$) lower for Sudani ducklings in SV4-group × T24th than those of the other experimental groups. This can be interpreted based on the increase of SV concentration could increase the amount of lymphocyte that improve antibody production, and hence the ducks immunity. Ismoyowati et al. (2012) and El-Kholy et al. (2020) stated that the fowl comfort may be indicated by measuring the H/L ratio. The H/L ratio is more liable as a fowl comfort than blood corticosterone level (Mohammadzade et al., 2013). McGrath et al. (2010) stated that fowl in a good welfare or normal physiological condition and thermoneutral zone is indicated with a lower H/L value than that of distress environment.

Blood serum constituents of Sudani ducklings were estimated to show the metabolic status of ducklings and their health as affected by in-ovo SV dipping. Concentrations of serum total protein and their fractions (albumin and globulin) and all lipid profile were insignificant affects by in-ovo different SV solutions, times of eggs dipping and their interaction. But concentrations of cholesterol, triglycerides and LDL were decreased numerically in SV3-group, while those of HDL were increased numerically. HDL has several bio-vital biological functions including (1) allowing hydrophobic lipid molecules like cholesterol and triglycerides to pass more easily through the water-based bloodstream; (2) cholesterol transfer to steroidogenic tissues such the adrenal gland, ovary, and testis; and (3) eliminating extra LDL molecules from the body via the liver. These facts may explain the decreased concentrations of triglycerides and total cholesterol (increased cellular uptake) obtained in the present study in SV3-group (Bernardo et al., 2000). Similarly, Agboola et al. (2015) reported that dietary supplementation of organic acids can reduce serum cholesterol of broiler chickens. It is accepted that inhibiting the absorption of dietary fat and fatty acid synthesis, and/or promoting fatty acid b-oxidation reduces serum total cholesterol by decreasing the size and/or the number of abdominal adipose cells.

As shown in Table 6, the glucose concentration numerically increased for ducklings produced from control and positive groups. Whereas the lowest concentration recorded for SV groups, this may be due to SV can buffer the acetyl CoA/ CoA ratio, thus preventing the negative feedback of high acetyl-CoA level on prolyl hydroxylase domain (PDH) activity. As a result, the breakdown of glucose can proceed, resulting in improved glucose utilization and hence decreased plasma glucose level (Feller and Rudman, 1988). In addition, during the latter days of incubation, a significant amount of energy is required to maintain the embryo's proper development. However, limited glucose supply in last time incubation of poultry embryos induces gluconeogenesis from amino acids produced by the degradation of protein of the breast muscle (Hamer and Dickson, 1989), which ultimately results in decreased protein deposition in breast muscle and organ weight decline (Vieira and Moran, 1999). Therefore, because of

significant energy catabolism, the final days of incubation and the first few days following hatching are important for the survival and development of embryos at the end of incubation and newborns in poultry (Salmanzadeh, 2012). Regarding to time of egg's dipping, the present study shows that significant increase in glucose concentration in T17th and T24th in compared to other experimental times. This result may be indicating the energy availability for different physiological and biochemical functions especially at 17th or 24th d of embryonic ages.

In-ovo SV dipping in any concentrations at T10th and T31st resulted in significant increases in serum-calcium concentrations in compared to other experimental groups (Figure 4). This improvement in serum-calcium concentrations by in-ovo SV may enhance calcium solubility and in turn improving intestinal calcium absorption. Furthermore, Kishi et al. (1999) observed that dietary vinegar improved intestinal calcium absorption by enhancing calcium solubility and the nutritional benefit of acetic acid contained in vinegar.

It is clearly noticed from the present results that, pre-incubation in-ovo dipping with different SV solutions improved significantly (P≤0.05) LBW, BWG, FCR and PI of Sudani ducklings at different ages from hatch to 21 days of age as compared to control groups. The best values of LBW, BWG, FCR and PI were recorded by the group dipping at 0.05% SV followed by groups dipping at 0.15 or 0.10 % SV throughout the experimental period, (from one up to 21 days of age). This may be a consequence of the high hatching weight of this group compared with the other groups. Moreover, the increased LBW, BWG, FCR and PI at 21st day is due mainly to the increased myogenesis during incubation because of SV dipping. Also, an increase in efficiency of fatty acids oxidation which subsequently led to an improved utilization of dietary nitrogen thereafter. Our results are in close agreement with (El-Naggar and Abo El-Maaty, 2017), on ducks and Zaib et al. (2016) and Rouzbeh et al. (2016) on broiler. Differently, Bhanja et al. (2012), Salary et al. (2014), and Rajkumar et al. (2015) did not find any improvement in newborn chicks from eggs with in-ovo SV supplementation. In contrast, some researchers have a positive assessed from dietary supplementation of vinegar (Allahdo et al., 2018). These differences are due mainly to different magnitudes of time and dose of SV and to the bird species, since in the present study Sudani ducks were used.

In the present experiment, however, better feed conversion was observed in ducklings from eggs dipping in SV, which indicates better use of the feed that was consumed. These findings corroborate the results that showed better development of the small intestine in birds supplemented in-ovo with SV. In study with acetic acid supplementation for broilers in the diet, Saleem et al. (2016) found better development to chicks' gut and better performance. In addition, it was also seen that chicks that received in-ovo SV, especially at the medium dose (2.5%), presented greater improvement in almost every chick quality variable studied. This may have been determinant for the better feed conversion rate presented by these ducklings throughout the first phase of rearing period studied. Lastly, the increase in ducklings weight at hatch may also have contributed to the improvement of overall performance.

According to Hudha et al. (2010), acetic acid supplementation in drinking water increased growth and feed conversion. Acetic acid is an organic acid that can limit the growth of microorganisms in the gastrointestinal system, modify pH levels, and improve feed utilization (Cooksley, 2011) which can explain improve of FCR in SV-groups compared to control group. Apart from lowering the pH of the intestines, acetic acid also stimulates and activates the pancreas, as well as the production of digestive enzymes, allowing more nutrients support the development of embryo's organs (Salahi et al., 2011). Also, dietary supplements such as organic acids have been shown to improve protein utilization and energy in poultry as mentioned by Yang et al. (2008), and Pirgozliev et al. (2008).

The production index, which is one of the best measurements of production efficiency of meat breeds, it takes into account of all live body weight, feed conversion ratio and duration of raising, It was noted from the Table VII that the values of the production index were improved in the SV dipping treatments which took the same improving path of live body weight and feed conversion ratio, it was significantly increased in SV2-group compared to other experimental groups. Interestingly, the highest PI value was belonging to the ducklings in SV2-group when eggs dipped at 10th d being 34.5±0.06. This result may be due to SV improves BWG and FCR as well as increases feed consumption during the initial rearing phase. Also, it may be due to SV can improve protein utilization and energy in poultry (Pirgozliev et al., 2008), which subsequently improved growth performance. These results are similar with those obtained by Awad et al. (2016) who reported that PI for Domyati ducklings was significantly improved by dietary natural supplementation. It seems that the literature is still sparse on the effect of in-ovo on PI value in ducks.

CONCLUSIONS AND RECOMMENDATIONS

In-ovo SV dipping improves newly hatched ducklings hemato-biochemical profile, thus enabling better early chick development and performance. Therefore, dipping

duck's eggs in 0.05% of SV solution, especially on day 10th of incubation is indicated for routine inoculation in industrial hatchery to improve the initial production of ducks. Studies are needed to evaluate the dipping of SV in-ovo on growth performance in the last part of production between 21 and 70 d.

NOVELTY STATEMENT

We found that in ovo feeding by dipping duck's eggs in 0.05% SV especially at 10th day of embryonic age could be contributed to the decrease of stress resulting from metabolic heat during the late period of hatching. Therefore, the in ovo dipping of 0.05% SV of sugarcane vinegar solution into the duck's eggs in day 10th of incubation may have a positive effect on the newly hatched ducklings hemato-biochemical profile, thus improvement of overall duckling's performance.

AUTHOR'S CONTRIBUTION

KHE, THT, SNS and AME developed the concept of the manuscript. All authors checked and confirmed the final revised manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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