

## Research Article



# Effect of in Ovo Dipping of Sugarcane Vinegar on Hatchability and Day Old Ducklings Quality

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**Abstract** | In-ovo dipping with exogenous materials, such as sugarcane vinegar (SV) solution, throughout incubation of duck's eggs could be a technique to boost hatchability and ducklings' quality. The objective of the present study was to determine the effect of in-ovo dipping of SV on the hatchability%, embryonic mortality, and internal and external 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 1<sup>st</sup> group without any treatment and served as a control (C), the 2<sup>nd</sup> group was dipped into distilled water as a vehicle or positive control (SV1). The 3<sup>rd</sup> group (SV2), the 4<sup>th</sup> group (SV3) and the 5<sup>th</sup> (SV4) group were dipped in 0.05, 0.10 and 0.15% of SV solutions, respectively, at four-time of eggs dipping: at day 10<sup>th</sup> (T10), day 17<sup>th</sup> (T17), day 24<sup>th</sup> (T24) and day 31<sup>st</sup> of incubation (T31) totaling ten groups with three replicates of (34 eggs of each). The results showed that the highest and lowest values of hatchability% and embryonic mortalities % were recorded for eggs dipped with 0.15% of SV solution for compared with other groups. The eggs which dipped at 24 d of time dipping recorded the best values for hatchability and embryonic mortalities % being the highest and the lowest, respectively as in compared to other experimental groups. Furthermore. There was no treatment × time interactions for the absolute and relative weights of duckling, liver and yolk sac, leg length, relative leg length, and yolk free body weight. However, such interactions were significant for hatchability and Tona Score. The highest values of leg length and duckling body weight/duckling length ratio produced from eggs dipped in nutritive solution were recorded at 17 and 31 d of embryonic ages, respectively. According to the results, it can be concluded that in-ovo dipping in 0.15% of SV solution, especially on day 24<sup>th</sup> of incubation has a positive effect on the hatchability%, embryonic mortality as well as quality of internal eggs and hatched duckling.

**Keywords** | Dipping, Duckling, Hatchability, In-Ovo, Sugarcane vinegar

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## INTRODUCTION

Day-old ducklings are the hatchery's final product and an important source of starting material for poultry farms. As a result, a high-quality day-old chick is an important link between the hatchery and the farm. On

the other hand, it has been found that good hatchability is not always associated to the highest post-hatch quality and growth of the chick, and that maximum hatchability is not always linked to the highest post-hatch quality and development (Decuypere and Bruggeman, 2007). Hatchability with many high-quality and saleable chicks

is one of the most important indexes in poultry industry (Decuypere and Bruggeman, 2007). The improvement of duck eggs hatchability is an important and critical input for lower the price of hatched ducklings at one day of age. The quality of the day-old chick has a significant impact on the poultry's growth performance (El-Kholy et al., 2018; 2020; 2021). Tona or Pascor score and day-old chick weight are commonly used for measuring chick quality (Tona et al., 2005). Chick weight is the most widely used indicator for day-old duckling quality assessment (Kleczeck et al., 2007). However, a better yolk uptake and a smaller yolk residue is an indicator of high-quality ducklings (Tona et al., 2005).

Nutrient deficiencies hinder the proper development of embryos, reduce hatchability, and increase embryonic death, in addition to causing disorders of the musculoskeletal system, immune system and cardiovascular system (Uni et al., 2012). The technique of early feeding of embryos by providing the incubated eggs with nutrients to support the fetus in the formation of body tissues and mitigate the stress during the hatching process (Ohta and Ishabish, 2001; El-Kholy et al., 2021). The nutrients in the egg determine the weight of the duckling at birth, body size and hatchability. Therefore, there are different methods used to improve the hatchability such as dipping eggs in nutrients during the incubation period (Abd El-Hack et al., 2019). Dipping eggs during the incubation period known as an in-ovo feeding method. Dipping eggs into nutritive solutions during the incubation period are considered the easiest method used to supply embryos with exogenous nutrient solutions (Ghonim et al., 2009; Al-Asadi and Ibrahim, 2020). In-ovo feeding is majorly a strategic nutritional intervention (El-Kholy et al., 2021). Shafey et al. (2012) and Tag El-Din et al. (2018) found that an in-ovo feeding embryos with nutrient solutions had improved hatchability percentage, and duckling's quality at hatch. Chick weight and shank length at hatch has great importance in poultry production for a good start of the chick and for the post hatch production performance (Gambo et al., 2014; Zhaoxiang et al., 2020).

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 acids and safe (Adil et al., 2010). It's a cheaper cost of the organic acid which used successfully in widespread medicine as sanitizer, antimicrobial, alleviating the effects of high environmental temperatures and reducing the stress of the chick looks during hatching.

Nowadays, information about the effects of in-ovo feeding by SV solution during the incubation period for duck eggs is lacking. Therefore, the objective of the current research was designed to study the appropriate embryonic age for dipping the fertile Sudani duck eggs and the optimum level of SV solution concentrate used to dip the eggs on hatchability percentage and hatched duckling's quality.

## 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.

### PREPARATION OF SUGAR CANE VINEGAR SOLUTIONS

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  $64 \pm 1$ g and distributed according to randomized block experimental design in a (5 × 4) factorial arrangement, consisting of five different SV solution concentrates; the 1<sup>st</sup> group without any treatment and served as a control (C), the 2<sup>nd</sup> group was dipped into distilled water as a vehicle or positive control (SV1). The 3<sup>rd</sup> group (SV2), the 4<sup>th</sup> group (SV3) and the 5<sup>th</sup> (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 10<sup>th</sup> (T10),

day 17<sup>th</sup> (T17), day 24<sup>th</sup> (T24) and day 31<sup>st</sup> of incubation (T31) totaling ten groups with three replicates of (34 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 incubation hall in 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 automatically every 1 h until they transferred to the hatching compartment at the 31<sup>st</sup> day of incubation. At 31<sup>st</sup> 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. Un-hatched eggs were examined the embryonic mortality and the hatchability of fertile eggs were calculated.

### DUCKLING QUALITY ASSESSMENT

Fifteen ducklings from each treatment were selected at random to individually weight to the nearest 0.1g and stretched along a ruler to measure length (cm) both the hatched duckling and right leg according to Lourens et al. (2006), then relative leg length (leg length × 100 / hatched duckling length ratio) value was calculated. The duckling body weight/ hatched duckling length ratio were calculated.

The ducklings were examined to identify the different characteristics that can be associated with quality duckling using Tona scoring system (Tona et al., 2003). According to this method, physical parameters including activity, down and appearance, eyes, conformation of legs, navel area, yolk sac, and remaining membranes and yolk were scored. The quality score for every duckling was defined as the sum of the scores assigned to all quality parameters.

A healthy duckling (saleable) was defined as being robust, clean, dry, and free from deformities (normal conformation of body), completely sealed navel, and no yolk sac or residual membrane protruding from the navel area (Tona et al., 2003). Percentage of healthy ducklings was calculated as the percentage of healthy duckling's quality to total hatched ducklings.

### STATISTICAL ANALYSIS

Data obtained were statistically analyzed using two-way analysis of using the General linear Model procedure of SAS (2012) as following model:

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

Where;

$Y_{ijk}$  = observed traits;  $\mu$  = 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 ( $\pm$ SEM). At  $P < 0.05$ , the statistical significance was recognized.

## RESULTS AND DISCUSSION

### HATCHING PERFORMANCE

Table 1 shows the data concerning the average of hatchability percentages and embryonic mortalities percentages, hatching weight, relative weight (%) and Tona score (%) due to dipping duck eggs with different concentrations of SV solutions at different time. There were significant ( $P < 0.05$ ) differences observed among the experimental groups about total hatchability, embryonic mortality and relative weight. The highest and lowest values of hatchability % and embryonic mortalities % were recorded for eggs dipped with 0.15% of SV solution for compared with other groups. It is interesting to note that dipped eggs with different concentrations of SV solution neither improved duckling hatching weight % or Tona score %.

Regrading to time of eggs dipping, Table 1 also shows that there were significant ( $P < 0.05$ ) differences observed among the experimental groups regarding hatchability percentages and embryonic mortalities percentages, hatching weight and relative weight (%). However, there were no significant ( $P > 0.05$ ) differences among the experimental groups for Tona score (%). It is interested to notice that the group which eggs were dipped at 24 d of embryonic age recorded the best values for hatchability and embryonic mortalities % being the highest and the lowest, respectively as in compared to other experimental groups.

There were marked effects on several duckling quality traits attributable to the SV treatment (Table 3). It is appeared that average of leg duckling length had ranged from 4.48 to 4.94 cm, where the highest leg duckling length recorded for ducklings hatched from eggs dipped in 0.15% of SV solution in compared with other experimental groups. On the other hand, there were insignificant differences observed among groups with respect to the duckling's quality traits including hatched duckling length, relative leg length and duckling body weight/duckling length ratio.

Regrading to time of eggs dipping, Table 1 also shows that there were significant ( $P < 0.05$ ) differences observed

**Table 1:** Effect of dipping fertile duck eggs into sugar can vinegar solution concentrates at different embryonic ages on hatchability traits and duckling quality.

Items	Hatchability (%)	Embryonic mortality (%)	Hatching weight (g)	Relative weight (%)	Tona score (%)
Overall mean	64.74	36.27	41.22	65.34	91.72
<b>Sugar can vinegar solution concentrate effect</b>					
C	58.56 <sup>c</sup>	41.15 <sup>a</sup>	40.36	63.69 <sup>b</sup>	91.60
SV1	66.41 <sup>b</sup>	33.59 <sup>b</sup>	40.93	65.91 <sup>a</sup>	93.90
SV2	60.24 <sup>c</sup>	39.76 <sup>a</sup>	41.50	65.87 <sup>a</sup>	92.10
SV3	67.53 <sup>b</sup>	32.47 <sup>b</sup>	40.72	65.31 <sup>ab</sup>	88.20
SV4	70.67 <sup>a</sup>	29.34 <sup>c</sup>	42.58	65.91 <sup>a</sup>	92.80
SEM	0.76	0.75	0.87	0.64	2.45
Sig.	**	**	NS	**	NS
<b>Embryonic age effect</b>					
EA10	61.04 <sup>c</sup>	38.96 <sup>b</sup>	42.44 <sup>a</sup>	66.15 <sup>a</sup>	91.68
EA17	68.06 <sup>b</sup>	31.94 <sup>c</sup>	39.03 <sup>b</sup>	67.50 <sup>a</sup>	89.84
EA24	75.97 <sup>a</sup>	24.03 <sup>d</sup>	40.13 <sup>b</sup>	63.89 <sup>b</sup>	93.36
EA31	53.89 <sup>d</sup>	46.11 <sup>a</sup>	43.26 <sup>a</sup>	63.81 <sup>b</sup>	92.00
MSE	0.67	0.67	0.78	0.58	2.19
Sig.	**	**	**	**	NS

a, b and c: means in the same column and effect bearing different superscripts are significantly different ( $P \leq 0.05$ ). SEM: standard error mean; NS: non-significant; \*:  $P \leq 0.05$ ; \*\*:  $P \leq 0.01$

**Table 2:** Effect of dipping fertile duck eggs into sugar cane vinegar solution concentrates at different embryonic ages on hatched duckling length, leg length, and relative leg length and duckling body weight: duckling length ratio.

Items	Hatched duckling length (cm)	Leg length (cm)	Relative leg length (%)	Body weight / duckling length
Over all mean	22.30	4.77	23.18	2.02
<b>Sugar can vinegar solution concentrate effect</b>				
C	19.86	4.58 <sup>b</sup>	23.04	2.03
SV1	30.51	4.93 <sup>a</sup>	22.55	1.91
SV2	20.33	4.80 <sup>a</sup>	23.55	2.05
SV3	20.13	4.62 <sup>b</sup>	22.89	2.03
SV4	20.67	4.94 <sup>a</sup>	23.85	2.06
SEM	4.36	0.06	0.55	0.06
Sig.	NS	**	NS	NS
<b>Embryonic age effect</b>				
EA10	20.64	4.94 <sup>a</sup>	23.89	2.06 <sup>ab</sup>
EA17	28.54	5.07 <sup>a</sup>	23.51	1.38 <sup>c</sup>
EA24	20.06	4.58 <sup>b</sup>	22.84	2.00 <sup>b</sup>
EA31	19.94	4.48 <sup>b</sup>	22.46	2.17 <sup>a</sup>
MSE	3.90	0.06	0.49	0.05
Sig.	NS	**	NS	**

a, b and c: means in the same column and effect bearing different superscripts are significantly different ( $P \leq 0.05$ ). SEM= standard error mean; NS: non-significant; \*:  $P \leq 0.05$ ; \*\*:  $P \leq 0.01$

among the experimental groups regarding leg length, and duckling body weight/duckling length ratio. However, there were no significant ( $P > 0.05$ ) differences among the experimental groups for hatched duckling length and relative leg length. The highest values of leg length and duckling body weight/duckling length ratio produced from eggs dipped in nutritive solution were recorded at 17<sup>th</sup> and 31<sup>st</sup> d of embryonic ages, respectively.

Data of duckling yolk sac weight, relative yolk sac, yolk free body mass, liver weight and relative liver weight at hatch are presented in Table 3. The lowest values of these parameters were recorded in eggs dipped in 0.15% of sugar cane vinegar solution in compared to other experimental groups.

Regrading to time of eggs dipping, Table 3 also shows that there were significant ( $P < 0.05$ ) differences observed



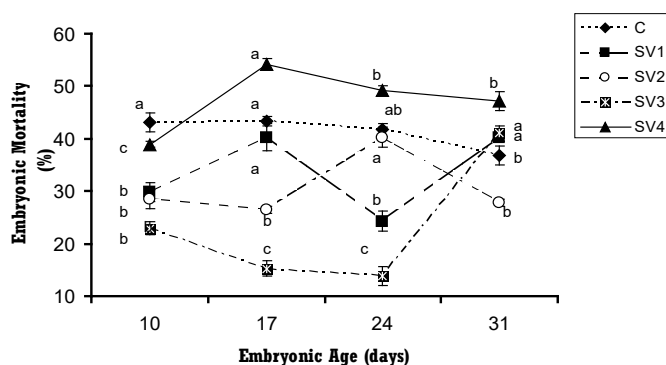
**Table 3:** Effect of dipping fertile duck eggs into sugar can vinegar solution concentrates at different embryonic ages on duckling yolk sac weight, relative yolk sac, yolk free body mass, liver weight and relative liver weight at hatch.

Items	Yolk Sac weight (g)	Relative Yolk Sac (%)	Yolk free body mass (g)	Liver weight (g)	Relative liver weight (%)
Overall mean	2.68	6.45	38.54	1.23	3.00
<b>Sugar can vinegar solution concentrate effect</b>					
C	3.14 <sup>a</sup>	7.74 <sup>a</sup>	37.23 <sup>b</sup>	1.23	3.07
SV1	2.68 <sup>ab</sup>	6.41 <sup>ab</sup>	38.25 <sup>ab</sup>	1.25	3.09
SV2	2.56 <sup>ab</sup>	6.14 <sup>ab</sup>	38.94 <sup>ab</sup>	1.21	2.93
SV3	2.68 <sup>ab</sup>	6.53 <sup>ab</sup>	38.05 <sup>ab</sup>	1.25	3.10
SV4	2.34 <sup>b</sup>	5.45 <sup>b</sup>	40.25 <sup>a</sup>	1.19	2.82
SEM	0.23	0.55	0.84	0.04	0.12
Sig.	**	**	**	NS	NS
<b>Embryonic age effect</b>					
EA10	3.29 <sup>a</sup>	7.77 <sup>a</sup>	39.15 <sup>a</sup>	1.15 <sup>b</sup>	2.72 <sup>b</sup>
EA17	2.34 <sup>b</sup>	6.01 <sup>bc</sup>	36.70 <sup>b</sup>	1.33 <sup>a</sup>	3.42 <sup>a</sup>
EA24	1.99 <sup>b</sup>	5.01 <sup>c</sup>	38.14 <sup>ab</sup>	1.29 <sup>a</sup>	3.23 <sup>a</sup>
EA31	3.07 <sup>a</sup>	7.02 <sup>ab</sup>	40.18 <sup>a</sup>	1.14 <sup>b</sup>	2.64 <sup>b</sup>
MSE	0.21	0.49	0.75	0.04	0.11
Sig.	**	**	**	**	**

a, b and c: means in the same column and effect bearing different superscripts are significantly different ( $P \leq 0.05$ ). SEM: standard error mean; NS: non-significant; \*:  $P \leq 0.05$ ; \*\*:  $P \leq 0.01$

among the experimental groups regarding duckling yolk sac weight, relative yolk sac, yolk free body mass, liver weight and relative liver weight at hatch. The lowest values for absolute and relative weights of yolk sac and liver produced from eggs dipped in nutritive solution were recorded at 24<sup>th</sup> and 31<sup>st</sup> d of time of eggs dipping, respectively.

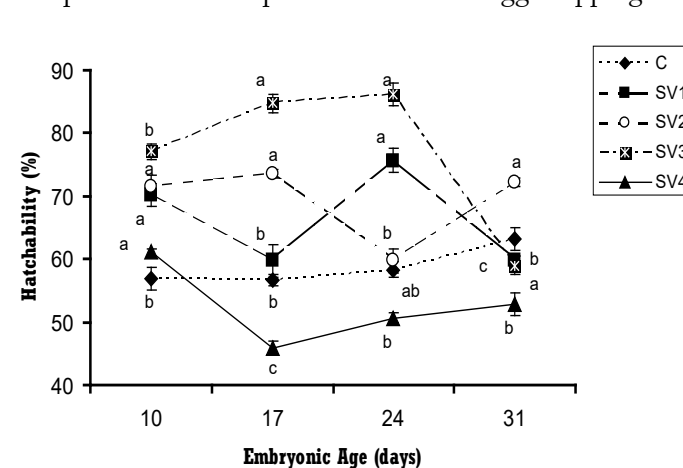
yolk sac, leg length, relative leg length, and yolk free body weight. However, such interactions were significant for hatchability and Tona Score (Figures 2 and 3). Ducklings hatched from SV4, SV3 and SV2 had significantly higher hatchability percentage on T24, T17 and T10 compared to T30, while these increases in SV1 ducklings were on T17 compared to other experimental time of egg's dipping.



**Figure 1:** Treatment  $\times$  time of eggs dipping interaction means ( $\pm$  SEM) for embryonic mortality. <sup>ab</sup> Means within times with unlike letters differ ( $P < 0.05$ )

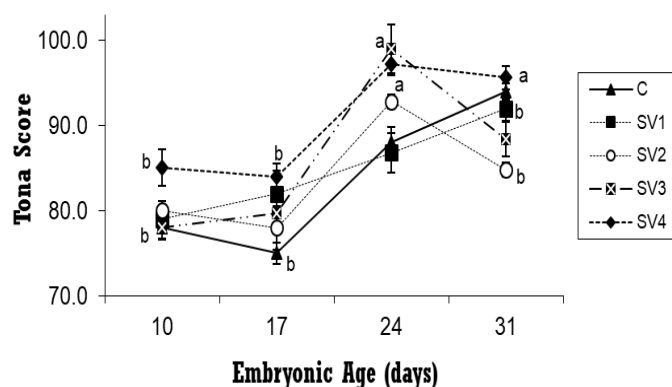
#### TREATMENT BY TIME OF EGGS DIPPING EFFECTS

Concerning of embryonic mortality was significantly affected by the treatment by time of eggs dipping interaction. The minimum embryonic mortality was observed ( $P < 0.05$ ) on T24 when embryonic mortality % was  $13.89 \pm 1.50$  in the SV4-group;  $15.28 \pm 1.50$  in the SV3-group;  $22.92 \pm 1.50$  in the SV2-group;  $27.78 \pm 1.50$  in the SV1-group versus  $40.28 \pm 1.50$  in the C-group ( $P < 0.05$ ) (Figure 1). There was no treatment  $\times$  time interactions for the absolute and relative weights of duckling, liver and



**Figure 2:** Treatment  $\times$  time of eggs dipping interaction means ( $\pm$  SEM) for hatchability %. <sup>ab</sup> Means within times with unlike letters differ ( $P < 0.05$ ).

Concerning of Tona Score was significantly affected by the treatment by time of eggs dipping interaction. The maximum Tona Score was observed ( $P < 0.05$ ) on T24 when Tona Score % was  $99.00 \pm 4.89$  in the SV3-group;  $97.20 \pm 4.89$  in the SV4-group;  $92.80 \pm 4.89$  in the SV2-group;  $86.80 \pm 4.89$  in the SV1-group versus  $88.00 \pm 4.89$  in the C-group ( $P < 0.05$ ) (Figure 3).



**Figure 3:** Treatment × time of eggs dipping interaction means (± SEM) for Tona score. <sup>ab</sup>Means within times with unlike letters differ (P<0.05).

During the incubation stage, duck eggs lose more than chicken eggs, either due to low fertility or low hatchability (Abd El-Hack et al., 2019). Ducks, overall, have a short breeding season and lay a small number of eggs. As a result, it's critical to keep these losses to a minimum to maximize the number of ducklings produced with the limited number of eggs available. On the other hand, the level of short-chain fatty acids in duck's caecum and intestine are very low at early period of life. Dipping eggs into nutritive solutions as in - ovo is new feeding strategy during the incubation period to supply embryos with exogenous nutrient solutions (Ghonim et al., 2009; Tag El-Din et al. 2018; Al-Asadi and Ibrahim, 2020).

In current study, improvement of hatchability % and reduction of duckling's embryos mortality in SV dipping treatments, may be because the process of SV dipping is an early feeding process, provides the embryo energy, organic acids as a direct source of energy for intestinal cells (Blank et al., 1999). Acetic acid (the most common organic acid found in SV) is also an intermediate component in cellular metabolism, where it is converted to Acetyl Co-enzyme A, which is then used in the Kreb's Cycle to produce energy ATP (Lehninger et al., 2012; Edan, 2018). On the other hand, the contents of short-chain fatty acids in SV (Chinnici et al., 2009) may be make up deficiency of short-chain fatty acids at early life of ducklings. Applegate (2002) demonstrated that providing embryos with energy by in-ovo during incubation increases hatching, because hatching is a stressful process for the embryo, and it requires more energy to complete it. Another study by Uni et al. (2017) demonstrated that in ovo with food solutions boosted hatching rates, because the embryo in the final period of embryonic development practically depletes the stored glycogen in the liver upon hatching, the body to get extra energy to pip the eggshell and exit when hatching. It's possible that the role of acetic acid in facilitating the process of breaking the shell during hatching through its interaction with calcium carbonate, which leads to

a lack of calcification of the crust and weak cohesion, facilitating the process of breaking the shell, is the reason for the superiority of acid injection treatments in terms of hatching rate (Uni et al., 2017). Also, hatchability in the present study may be improved due to decreasing the embryonic mortality, where SV may be considered as an anti-stress agent. Sugarcane vinegar contains polyphenols and flavonoids compounds that act as strong antioxidants for reducing oxidative stress (Li et al., 2020).

These results were agreed with Ghonim et al. (2009), Yassein and Beamish (2014) and Al-Hamed and AL-Eshaki (2019). On the other hand, Al-Asadi and Ibrahim (2020) showed that the application of immersion did not affect embryonic mortality.

Interestingly, these results were significant at 24<sup>th</sup> d of eggs dipping in either 0.10 or 0.15% of SV solutions. This may be due to the increased stress at late of embryonic age which may have increased the biological requirements from beneficial components of SV solutions. The marked changes observed in percentages of hatchability and embryonic mortality reflected the ability of SV to mitigate the harmful effects of incubation stress, facilitating more healthy duckling.

The embryo's development and growth throughout the incubation phase is entirely dependent on the egg components (albumin, yolk and eggshell). In this study, a significant decrease in the yolk sac and increase of yolk free body mass were observed at SV treated eggs especially at day 24<sup>th</sup> and 31<sup>st</sup> of dipping time, respectively (Table 2). The same trend was found in the chicks after hatch which reflect the beneficial effects of organic acids on protein utilization (Yang et al., 2008; Pirgozliev et al., 2008). Yolk residual relative weights from the same treatments were lower than control group. Our results agreed with those of Rouzbeh et al. (2016) and Fouad et al. (2019).

It was noted from Table 3 that the relative body weight of hatched ducklings was improved by dipping eggs in the Acetic acid from which it was hatched, the dipping SV treatments for at the highest levels were significantly higher (P≤0.05) in this characteristic compared to control treatment. The current study also noted that the body length of the hatched chicks was significantly higher (P≤0.01) in the SV dipping eggs compared to control one.

Our results showed an improvement in the internal duck's egg quality, also the weight of the ducklings at hatch was significantly higher for eggs treated with SV (Tables 2 and 3). These improvements may be due to the enhancement health status of embryos, because of treatment with natural SV solution. Kirchgessner and Roth (1988) reported that acidification with various weak organic acids such

as vinegar improve digestibility of protein and of P, Ca, Mg and Zn and acts as substrates in the intermediary metabolism and reduces the colonization of pathogens and the production of toxic metabolites. An organic acid such as acetic acid has been used in diets because of their positive effect on health and bird growth. The use of organic acids has become more acceptable as in ovo. Immersion treatments resulted in an increase weight of chicks and the ratio of the chick's weight to egg weight, this increase may be due to the sugarcane vinegar contain acetic acid (used in the Krebs'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.

The lowest and highest values of absolute and relative weights of yolk sac and ducklings and leg length, respectively, were recorded in eggs dipped in 0.15% of sugar cane vinegar solution (SV4). These results are in agreement of the findings of Al-Azzawi et al. (2018). 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 from the yolk sac to be absorbed (which weighs less) and aids the development of embryo's organs (Salahi et al., 2011), this is consistent with Lourens et al. (2006) who confirmed that there is a high correlation between the length of day-old hatched chick with its zero yolk mass and the negative correlation between the residual yolk and its yolk-free mass. So, the consumption of yolks early may be gives better results to the ducklings because the remaining yolk contains all the nutrients of fat, amino acids, vitamins, and minerals needed by the embryo for its growth and development.

The improvement in the characteristics of the ducklings, which represents in increased tona score and relative weigh in acid dipping eggs treatments may be related to acetic acid's influence on embryos, which improves her gut's growth, development, and activity while also promoting nutrients uptake from the yolk sac, Uni and Smith (2017) indicated that chicks embryos have the ability to digest and absorb nutrients before hatching but the intestinal function begins only at the time the embryo intake the fluid that surrounds it through the mouth at the age of 17-19 days of incubation, so the growth of intestinal tissue, maturation and metabolism become of great importance in the recent period on the growth and development of the embryo, the feeding of embryos. In ovo feeding (IOF) with acetic acid in the amniotic fluid during this period will be absorbed by the enterocytes and will be a direct source of energy for these cells (Scheppach et al., 1995).

Among the reasons that led to an increase in chick length and leg length in SV dipping groups are the

increase in the formation of nutrients during the period of embryonic development and benefit from them that are carried in the cane solution. Where studies showed a positive relationship between the chick's length and productive performance (Michalczuk et al., 2011). Due to the presence of the residual non-absorbed yolk that is calculated with chick weight, chick length is a stronger indicator of future performance than chick weight (Mukhtar et al., 2013; Patbandha et al., 2017). These results were consistent with what Fouad et al. (2019) and Al-Asadi and Ibrahim (2020) who observed significantly increased chick length by using in ovo either by injection or dipping with nutrients solutions. The reason for leg length may be due to the cane solution containing a high amount of minerals and trace elements such as calcium, phosphorus, manganese, zinc, and iron (> 9 mg/kg) (Xiong et al., 2014), it was confirmed by the results obtained by Salary et al. (2017), who concluded that injecting different levels of calcium enabled them to accelerate the growth, maturity, and development of bone cells.

## CONCLUSIONS AND RECOMMENDATIONS

As found in current results. the in ovo feeding by dipping duck's eggs especially at 24<sup>th</sup> day of embryonic age could be contributed to the decrease of stress resulting from metabolic heat during the late period of hatching. Therefore, in- ovo by SV may be useful for the vitality of the embryos during the incubation period and enhance day-old duckling quality.

## NOVELTY STATEMENT

We found that in ovo feeding by dipping duck's eggs in 1.15% SV especially at 24<sup>th</sup> 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 1.15% SV of sugarcane vinegar solution into the duck's eggs during the last days of incubation may have a positive effect on the hatchability%, embryonic mortality as well as quality of internal eggs and day-old duckling

## 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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