

EFFECT OF ASCORBIC OR CITRIC ACID SOLUTIONS ON EGG SHELL CONDUCTANCE AND INCUBATION TRAITS OF SINAI EGGS.

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ABSTRACT

The present experiment was conducted to study the effects of organic acids (Ascorbic and Citric) on egg weight loss, hatching performance and embryonic mortality in fertile eggs from Sinai hens.

Results showed that :

- Ascorbic acid (10gm/L) dipping treatment for 1 minute (AA1) significantly increased hatchability percentage and significantly decreased all rates of embryonic mortality.
- Ascorbic acid (10 gm/L) dipping treatment for 2 minutes decreased significantly the percentage of culled chicks.
- Citric acid (20 gm/L) dipping treatment for 2 minutes (CA4) had significantly the highest averages of egg weight loss, albumen and amniotic fluid pH, culled chicks also early and late embryonic mortality. while it decreased albumine height embryo weight at the 14th day of incubation period and hatchability percentage.
- Citric acid (10gm/L) dipping treatment for 1 minute increased significantly embryo weight at 14th day of incubation period.
- Distilled water dipping treatments increased significantly the rate of piped dead embryos however, it decreased egg weight loss and albumin pH.
- Non-dipped eggs had significantly the highest rates of albumin height, chick's weight at hatch and unhatched eggs with piped live embryos but recorded the lowest value of amniotic pH.

It is concluded that, dipping eggs in ascorbic acid solution at a concentration of 10 gm /L for 1 minute increased significantly hatchability percentage and decreased embryonic mortality.

EINTRODUCTION

The structure of hen's eggshell was studied during the incubation. The main biological function of the eggshell in the domestic fowl is to provide an environment in which a new chick can develop by allowing for adequate movement of water vapour and respiratory gases (Tullett, 1978). On the external surface of the shell there is a homogenous thin layer (the cuticle). It remains changeless during the whole period of incubation (Dorskocil *et al.*, 1985). The cuticle is placed on the shell by the hen, moments before the egg is laid. It is a liquid protein coat that dries within minutes after lay. Cuticle is usually deposited on hen's egg to a depth of 0.005 to 0.010 mm. (Romanoff and Romanoff, 1949).

The main part of shell is formed by long prisms with their long axis oriented perpendicularly to the external surface of the shell. These prisms are partially twisted round each other, similarly as in the case of the teeth (Dorskocil *et al.*, 1985).

The eggshell is consists of an overlaying cuticle, a crystalline calcium carbonate layer with tow underlying proteinaceous membranes (Taylor, 1970)

and is penetrated by thousands of microscopic pores, which are essential for the exchange of respiratory gases during incubation (Rhan and Pagnelli, 1979). The cuticle may bridge the pore openings or extend down into them to form a plug (Board, 1982). This may cause a restriction on the exchange of gases between the embryo and the air surrounding the eggs. The resistance to this exchange of gases has been attributed to the shell proportion of calcium carbonate, shell membranes and cuticle (Tullett, 1978 and Rhan and Pagnelli, 1979). Treatment of the shell with weak acids release carbon dioxide (Romanekewitsch, 1934), enhance the movement of water vapour, change the buffering capacity of albumin and influence hatchability percentage of eggs (Meuer and Baumann, 1988; Burley and Vadehra, 1989). Moreover, albumin pH, embryonic mortality, culling chicks and chick's weight at hatch were also affected (Shafey, 2002). Ascorbic acid is an organic acid and the ability of diluted acids to interact with the eggshell cuticle was reported by (Burley and Vadehra, 1989). The aim of this study was to determine the effect of dipping eggs in different dilution of organic acids ascorbic and citric acids for different time on egg weight loss, albumin pH, hatchability, hatching weight and embryonic mortality.

MATERIALS AND METHODS

The present study was carried out at Gemmizah Poultry Research Station, Animal Production Research Institute, Agriculture Research center, Ministry of Agriculture, Egypt. A total number of 1100 fertile, eggs were obtained from Sinai local strain hens which were fed on a layer ration containing (16 % crude protein, and 2730 kcal ME/ kg of diet). Birds were reared under similar hygienic and environmental conditions. Ascorbic and citric acid solutions were freshly prepared, dissolved in distilled water and kept in sealed bottles and protected from light. Large glass beakers were used for dipping eggs in dark room at room temperature.

A total number of 1100 fertile eggs (with approximately similar weights 42.3 ± 0.1 gm) were used in this study. Eggs were randomly divided into eleven groups, 100 eggs each as follows: non-dipped control (CND); water dipped (CWD) for 1 minute and 2 minutes (CWD1 and CWD2); 10gm ascorbic acid /L for 1 minute and 2 minutes (AA1 and AA2); 20 gm ascorbic acid/ L for 1 minute and 2 minutes (AA3 and AA4); 10 gm citric acid/ L for 1 minute and 2 minutes (CA1 and CA2); 20 gm citric acid /L for 1 minute and 2 minutes (CA3 and CA4), respectively.

Eggs were weighed individually, then immediately dipped into the experimental solutions and allowed to dry at room temperature. After dipping five eggs were randomly taken from each treatment to determine albumin pH and height.

Eggs were set in a Reform, force-draft incubator and incubated at 37.6°C and 55% humidity until they were transferred to the hatchery (37.2°C and 65% humidity) at the 19th day of incubation.

During the incubation period, eggs were examined by candling at the 6th and 12th days of incubation, where, infertile eggs and eggs containing

dead embryos were removed. Early dead embryos were counted from 1 to 12 day of incubation. At the 14th day of incubation 5 eggs were randomly chosen from each treatment for the determination of amniotic fluid pH and embryo weight (the embryo separated from the yolk sac, blotted dry with tissue paper and weighed to the nearest 0.01 gm), thereafter, at the morning of the 19th day of incubation, eggs were weighed individually before transferring to the hatchery, then egg weight loss was calculated as a percentage of the initial egg weight.

At the morning of the 22nd day of incubation, cull chicks, pipes (unhatched eggs with live or dead chicks) and late dead embryos (unhatched eggs with unbroken shell from the 12th to 22nd day of incubation) were counted and expressed as percentages from the fertile eggs. Hatchability was calculated as the percentage of hatched chicks from the fertile eggs per treatment. Culling of chicks was based on the general appearance of them, where, the small, weak and chicks with rough feathers were culled.

Data collected were subjected to analysis of variance as one way ANOVA, using the statistical analysis of (SPSS, ver 10,1997) and differences between treatment means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1- Egg weight loss , albumin pH and height:

Data presented in Table (1) showed that, dipping treatment in 20 gm/L CA for 2 minutes increased significantly egg weight loss (16.26%), albumin pH (9.22) and decreased significantly albumin height (4.32 mm), followed by those dipped in 20 gm/L of AA for 2 minutes (16.08%, 9.20 and 4.78 mm, respectively), while, the lowest egg weight loss value and albumin pH were recorded by those of CWD2 and CWD1 (12.61 and 8.10, 12.60 and 8.10 ,respectively). CND treatment had the highest value of albumin height (6.04 mm), followed by those of CWD1 (6.00 mm). In general, dipping eggs with CA had the highest values of egg weight loss and albumin pH (15.51% and 8.88, respectively), on the other hand, it decreased albumin height (5.32 mm) when compared to those dipped in AA or those of CWD.

The concentration of 20 gm/liter from both AA and CA acids increased the rate of egg weight loss (15.93 %) and albumin pH (9.08), however it decreased albumin height (4.97 mm) compared with those dipped in 10 gm /L (14.98 , 8.84 and 3.72 respectively).

Dipping period showed highly effect on egg weight loss, albumin pH and height. Dipping period for 2 minutes increased egg weight loss and albumin pH, while it decreased albumin height compared with those dipped for 1 minute.

These results agreed with those of Shafey, (2002) who reported that eggs dipped into 20 or 30 gm /L of ascorbic acid produced a greater increase in egg shell conductance, higher albumin pH and decreased albumin height . This may be attributed to the change in the cuticle properties obtained from the action of citric and ascorbic acids, which affect the morphology of cuticle

and produce a thinner cuticle, then, egg shell conductance will increased. Increasing eggshell conductance will enhance the movement of water vapour and carbon dioxide across the shell (Burley and vadehra, 1989). Increasing gas exchange across the shell will reduce carbon dioxide and alter albumin pH.

Table(1) : Means and standard error of egg weight loss, albumin height and pH for different experimental groups as affected by Ds, C and DT

Trait Treatment	Egg weight loss (%)	Albumin height (mm)	Albumin pH
CND	1390 ⁱ ± 0.01	6.04 ^a ± 0.02	8.38 ^d ± 0.05
CWD1	12.60 ^j ± 0.01	6.00 ^{ab} ± 0.10	8.10 ^e ± 0.05
CWD	12.61 ^j ± 0.01	5.94 ^{ab} ± 0.12	8.10 ^e ± 0.05
AA1	14.80 ^g ± 0.01	5.94 ^{ab} ± 0.12	8.10 ^e ± 0.03
AA2	15.10 ^f ± 0.01	5.64 ^{bc} ± 0.05	8.50 ^d ± 0.03
AA3	15.60 ^d ± 0.01	5.49 ^{cd} ± 0.03	8.90 ^{bc} ± 0.03
AA4	16.08 ^p ± 0.02	4.78 ^e ± 0.06	9.20 ^a ± 0.03
CA1	14.50 ^h ± 0.07	5.81 ^b ± 0.03	8.50 ^d ± 0.06
CA2	15.51 ^e ± 0.03	5.49 ^{cd} ± 0.06	8.80 ^e ± 0.03
CA3	15.78 ^c ± 0.03	5.30 ^d ± 0.03	9.00 ^b ± 0.03
CA4	16.26 ^a ± 0.02	4.32 ^f ± 0.10	9.22 ^a ± 0.10

a,b,c,... Means within columns with different superscripts are significantly different (P<0.05)

2- Developing embryo:

2-1-Embryo weight % at 14 days of incubation (as a percent from egg weight):

Data presented in Table (2) showed that, eggs that dipped in 10 gm /L citric acid for 1 minute had the highest embryo weight % at the 14th day of incubation period (23.42 %) when compared with other treatments or non – dipped, control (22.45%). On the other hand, eggs that were dipped for 2 minutes in a solution of 20 gm /L citric acid or ascorbic acid recorded significantly the lowest values for embryo weight (20.11 and 20.20%), respectively. This may be attributed to the higher increases in egg shell conductance of eggs dipped in 20 gm /L of citric or ascorbic acid which caused great water loss during the incubation period. These results agreed with those of Rhan and Pagnelli, (1980) who noted that, the rate of embryonic growth is related to the rate of water loss during the incubation period which.

Generally, eggs which were dipped in distilled water had the highest relative value of embryo weight (22.67%) compared with those of eggs dipped in either ascorbic or citric acids (21.37 and 21.13% respectively). Embryo weight percentage of eggs dipped in either ascorbic or citric acid at a concentration of 10 gm /l were higher than for those dipped at 20 mg / L (22.19 vs 20.32%) .Dipping eggs in either ascorbic or citric acid for 1 minute increased relative weight of embryo (21.94 %) more than those dipped for 2 minutes (21.3 %).

2-2- Amniotic fluid pH:

Data listed in Table (2) showed that, amniotic fluid pH recorded significantly its lowest value in non- dipped, control eggs (6.7) when compared with eggs of different treatments applied. On the other hand, the highest value was recorded in eggs dipped in citric acid solution at a concentration of 20 gm/L for 2 minutes (7.32) followed by those dipped in ascorbic acid solution at a concentration of 20 gm/L for 1 or 2 minutes, respectively (7.31 and 7.30).These results agreed with those of Hoyt (1979) who found that embryos tolerated different rates of water loss from their eggs during incubation by varying the amount of water absorbed from the allantoic fluid. Eggs dipped in distilled water had the lowest value (6.82)of amniotic fluid pH compared with those dipped in ascorbic acid (7.11) or citric (17.06) solutions.

Table (2): Means and standard error for developing embryo and hatching performance of different experimental groups as affected by studied factors

Treatment	Developing embryo		Hatching performance		
	Embryo weight (%)	Amniotic fluid pH	Hatchability (%)	Hatching weight (gm)	Culled chicks (%)
CND	22.45 ^c ± 0.06	6.70 ^c ± 0.03	83.54 ^d ± 0.16	33.72 ^a ± 0.06	2.40 ^d ± 0.03
CWD1	22.45 ^c ± 0.04	6.80 ^c ± 0.10	85.90 ^c ± 0.49	31.22 ^c ± 0.13	3.32 ^d ± 0.03
CWD2	22.88 ^b ± 0.05	6.85 ^{bc} ± 0.02	85.89 ^c ± 0.09	31.50 ^c ± 0.16	2.40 ^d ± 0.03
AA1	22.88 ^b ± 0.05	6.85 ^{bc} ± 0.02	91.20 ^a ± 0.09	31.50 ^c ± 0.16	1.00 ^g ± 0.09
AA2	21.92 ^d ± 0.07	7.31 ^a ± 0.06	88.31 ^b ± 0.09	31.65 ^c ± 0.13	0.12 ⁱ ± 0.01
AA3	20.50 ^e ± 0.07	6.97 ^{bc} ± 0.04	82.20 ^e ± 0.09	30.46 ^d ± 0.29	1.34 ⁱ ± 0.01
AA4	20.20 ^f ± 0.03	7.30 ^a ± 0.10	78.00 ^h ± 0.01	29.42 ^e ± 0.16	3.18 ^b ± 0.01
CA1	23.42 ^a ± 0.02	6.88 ^{bc} ± 0.04	88.48 ^b ± 0.09	32.21 ^b ± 0.02	1.50 ^e ± 0.01
CA2	20.54 ^e ± 0.04	6.92 ^{bc} ± 0.10	84.06 ^d ± 0.01	32.12 ^b ± 0.12	0.52 ^h ± 0.03
CA3	20.46 ^e ± 0.04	7.10 ^{ab} ± 0.04	79.52 ^g ± 0.09	30.14 ^d ± 0.05	2.80 ^c ± 0.06
CA4	20.11 ^f ± 0.07	7.32 ^a ± 0.20	71.64 ⁱ ± 0.09	29.23 ^e ± 0.07	4.32 ^a ± 0.01

a,b,c,... Means within columns with different superscripts are significantly different (P<0.05)

3- Hatching performance:

3-1- Hatchability percentage:

Hatchability percentage reached significantly its highest value in eggs dipped in 10 gm /L ascorbic acid solution for 1 minute (91.20 %) followed by those dipped in citric acid at the same previous concentration and time (88.48 %), while the lowest value was recorded in eggs dipped in 20 gm /L citric acid for 2 minutes (71.64 %) as shown in Table (2). These results agreement with those reported by Shafey (2002) who noted that, eggs dipped in 10 gm/L ascorbic acid for a period of 2 minutes improved hatchability percentage. He added that, this might be created the best internal environment for the embryo to hatch successfully. In average dipping substance had great effect on hatchability percentage, where, eggs dipped in distilled water had the highest percentage of hatchability (85.90%) followed by those dipped in ascorbic acid (84.93%) then those dipped in citric acid (80.93).Also hatchability percentage decreased when concentration of dipping solution or dipping period decreased for 88.01 to 77.84 and 85.35 to 80.50 respectively .

3-1- Hatching weight:

Data presented in Table (2) showed simply that, Non- dipped control eggs had significantly the highest chick's weight at hatch (33.72 gm) followed by those hatched of eggs dipped in citric acid solution at a concentration of 10 gm/L for 1 or 2 minutes (32.21 and 32.12 gm, respectively) compared with other treatments applied.

These results agreed with the fact that, the rate of embryonic growth is related to the rate (31.87 vs 29.31 and 31.11 vs 30.79) of water loss from the incubated egg (Rahn and Pagnelli, 1980). Shafey (2002) reported also that, the larger increases in eggshell conductance of eggs dipped in 20 or 30-gm/L ascorbic acid caused greater water loss during incubation.

3-2- Culled chicks:

Eggs dipped in 10 gm /L ascorbic or citric acid for 2 minutes had significantly the lowest culled chicks percentages (0.12 and 0.52 %, respectively) compared with other dipping treatments. However, dipping eggs in 20 gm /L citric acid for 2 minutes had significantly the highest value of culled chicks percentage (4.32%). It was obviously clear from these results that, culled chicks rate was increased by the increasing of solution concentration . In average ascorbic acid dipping treatment recorded the lowest value of culled chick's percentage when compared with those dipping in either citric acid (2.14) or distilled water (2.36). Also the concentration of 10 gm /L decreased the percentage of culled chicks (1.14) when compared with 20 gm / L (2.19). On the other hand, culled chicks percentage decreased from 2.36 to 1.80 when dipping period decreased from 2 to 1 minute .

4- Embryonic mortality (%):

4-1- Early dead embryos:

Data presented in Table (3) showed that, eggs dipped in 10 gm/L ascorbic acid for 1 minute significantly decreased early dead embryos (2.97 %) when compared with other dipping treatments or non-dipped control. This may be due to an increase in albumin pH and a reduction in albumin height. These changes in albumin might be due to the increase eggshell conductance of eggs and consequently the carbon dioxide from the egg, as the increase in liquefaction of albumin reduces the barrier to gaseous diffusion imposed by albumin. The absence of albumin liquefaction may increase the possibility of early embryonic mortality (Meuer and Baumann, 1988) Generally citric acid dipping treatment had the highest percentage of early dead embryo (4.97 %) compared with ascorbic acid treatment (4.87%) or distilled water-dipping treatment (4.73 %).Also the percentage of early dead embryo was increased by the increasing either concentration or dipping period (from 3.51 to 6.33 and 4.60 to 5.24 respectively) .

4-2- Late dead embryos:

Eggs dipped in 20 gm /L citric acid for 2 minutes recorded significantly the highest percentage of late dead embryos (16.38 %),Table (3) . Dipping eggs in 10 gm /L ascorbic or citric acid for 1 minute and distilled water for 2 or 1 minute decreased significantly late dead embryos (3.46,3.48, 3.50 and 3.70 %, respectively). These results agreement with those reported by Davis and

Ackerman (1987) and Shafey (2002) who stated that, the rate of water loss from eggs treated with 20 or 30 gm ascorbic acid/L may have been increased above the optimum range of water loss required for the growth and survival of the embryo at the late stage of incubation. In general distilled water dipping treatment had the lowest late dead embryo percentage (3.60) concentrated with other dipping treatments (6.91) for AA and 9.21 for CH . It is obviously clear that, late dead embryos percentage was affected positively with solution concentration or dipping period. It increased from 4.15 to 4.61 and from 6.10 to 10.02 by increasing solution concentration and dipping period respectively.

4-3- Pipes with dead or live embryos:

Data listed in Table (3) showed that, eggs dipped in 10 gm /L ascorbic acid for 1 minute decreased the percentages of pipes with dead and live embryos (1.69 and 0.68 % , respectively) when compared with other dipping treatments or non-dipped, control .

Generally, ascorbic acid dipping treatment had the lowest percentages of pipes with dead and live embryos (2.84 and 0.80 respectively) when compared with other dipping treatments. Concentration of 10 gm /L improved percentage of pipes with dead and live embryos from 3.22 to 2.65% and from 1.34 to 0.99 % respectively . Also dipping period for 1 minute improved the two studied traits from 3.23 to 2.64 % and from 1.35 to 0.98 % respectively .

Table (3): Means and standard error for embryonic mortality (%) of different experimental groups as affected by studied factors

Trait Treatment	Embryonic mortality (%)			
	Early dead embryos	Late dead embryos	Pipes with dead embryos	Pipes with live embryos
CND	5.52 ^e ± 0.03	5.07 ^f ± 0.20	2.45 ^b ± 0.11	3.42 ^a ± 0.10
CWD1	4.95 ^f ± 0.01	3.70 ⁱ ± 0.10	3.53 ^a ± 0.30	1.92 ^c ± 0.10
CWD2	4.51 ^g ± 0.06	3.50 ^j ± 0.01	3.37 ^b ± 0.01	2.73 ^b ± 0.01
AA1	2.97 ^j ± 0.06	3.46 ^j ± 0.01	1.69 ^c ± 0.01	0.68 ^f ± 0.01
AA2	3.82 ^h ± 0.06	3.92 ^h ± 0.01	3.23 ^a ± 0.01	0.72 ^f ± 0.01
AA3	6.15 ^c ± 0.01	7.65 ^d ± 0.01	3.22 ^a ± 0.03	0.78 ^f ± 0.01
AA4	6.53 ^b ± 0.01	2.60 ^b ± 0.01	3.23 ^a ± 0.03	1.00 ^e ± 0.01
CA1	3.45 ⁱ ± 0.06	3.48 ^j ± 0.01	2.45 ^b ± 0.03	0.78 ^f ± 0.07
CA2	3.78 ^h ± 0.03	7.18 ^e ± 0.01	3.22 ^a ± 0.03	1.76 ^d ± 0.01
CA3	5.83 ^d ± 0.06	9.80 ^c ± 0.02	3.19 ^a ± 0.03	1.66 ^d ± 0.01
CA4	6.81 ^a ± 0.06	6.38 ^a ± 0.02	3.25 ^a ± 0.01	1.92 ^c ± 0.01

a,b,c,... Means within columns with different superscripts are significantly different (P<0.05)

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تأثير محلول حمض السكوريك او الستريك على نفاذية القشرة و صفات التفريخ في بيض سلالة سيناء.

هشام رجب أمين سمك و رمضان مغاوري محمود على
معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية- الجيزة- مصر.

- اجري هذا البحث لدراسة تأثير الأحماض العضوية (حمض الأسكوريك و الستريك) على معدل فقد في وزن البيضة و نسبة الفقس و النفوق الجنيني و كانت النتائج كالتالي:-
- سجلت معاملة الغمر في محلول حمض الأسكوريك بتركيز ١٠ جرام / اللتر لمدة دقيقة أكبر تأثير معنوي على نسبة الفقس كما إنخفض معدل النفوق الجنيني معنويا.
 - سجلت المعاملات بالغمر في محلول الأسكوريك بتركيز ١٠ جرام / اللتر لمدة دقيقتين انخافض معنوي في نسبة الكتاكيت المستبعدة .
 - كان الغمر في محلول حمض الستريك بتركيز ٢٠ جرام / اللتر لمدة دقيقتين تأثير معنوي في زيادة معدل فقد من وزن البيضة و درجة تركيز أيون الهيدروجين لكل من الألبومين و السائل الأمنيوي و إنخافض معنوي في ارتفاع الألبومين ووزن الجنين عند اليوم الرابع عشر من التفريخ و معدل الفقس.
 - كان للغمر في محلول حمض الستريك بتركيز ١٠ جرام / اللتر و لمدة دقيقة تأثير معنوي في زيادة وزن الاجنة في اليوم الرابع عشر من التفريخ.
 - سجل الغمر في الماء المقطر أعلى معدل للبيض ذو الاجنة الناقرة الميتة بينما إنخافض معنويا كل من معد الفقد من وزن البيضة و تركيز أيون الهيدروجين في الألبومين.
 - سجل البيض الغير معنويا أعلى القيم في ارتفاع الألبومين ووزن الكتاكيت الفاقسة و عدد البيض الغير فاقس الذي يجوي اجنة ناقرة حية بينما إنخافضت درجة تركيز أيون الهيدروجين في السائل الأمنيوي.
 - و قد خلصت الدراسة الى ان غمر البيض في محلول حمض الأسكوريك بتركيز ١٠ جرام / اللتر لمدة دقيقة أدى معنويا الى زيادة نسبة الفقس و انخافض معدل النفوق الجنيني.

