Eastern Journal of Agricultural and Biological Sciences (EJABS)
ISSN: 2753-3247
Website: https://qabasjournals.com/index.php/ejabs

Bio Buffers as anew tools for elongation storage period of hatching eggs

Osamah Mohammed Abdullah
Department of Animal Production, College of Agricultural Engineering Science, University of Baghdad Iraq.
Corresponding Author: Author's Name, Osamah Mohammed Abdullah E-mail: osamah1986@yahoo.com

ARTICLE INFO

Received: May 15, 2022
Accepted: June 14, 2022
Volume: 2
Issue: 2

KEYWORDS

Elongation storage period, In ovo injection, HEPES, Tris, Bicine, Bis-Tris-Propahe (BTP)

ABSTRACT

Recent studies seek to find alternatives for prolonging the storage period of hatching eggs, through which it is possible to reduce the unfavorable effects of storage and increase the pH value. Therefore, researchers tended to the process of injecting hatching eggs with biological buffers to enhance the ability of eggs albumen to regulate the pH value in storage to be appropriate for embryo development. The process of injecting hatching eggs (in ovo injection) is one of the early feeding techniques for the embryo inside the egg for obtaining chicks of a high ability of production. Among the injectable buffers are HEPES, Tris, Bicine, and Bis-Tris-Propane (BTP).

1. Introduction The hatching eggs produced by broiler breedes are the core of the poultry industry's development due to their importance in Multiplying chicks and providing white meat. With the development of this industry, the demand for good quality hatching eggs increased to enhance the hatchability percentage and produce chicks with chick qualities. Thus, storing hatching eggs became one of the necessary and prevalent processes in the fields of Broiler breeding and commercial hatcheries (Fasenko, 2007).

Storing eggs for a long time results in many problems leading to the deterioration of the egg's internal quality and thus leading to a decrease in the hatchability and death of embryonic cells in the early stages, as well as the loss of moisture from inside the egg during the storage process and consequently to a change in the pH value of the egg as well as a decrease in albumen viscosity (Meijerhof, 1992). Storing hatching eggs for up to 21 days leads to a decrease in hatchability from 91% to 71% (Dymond et al., 2013). Bakst et al. (2012) showed also that embryonic cells die inside the egg after a period of up to 10-12 days of storing hatching eggs.

Many studies have sought to find appropriate solutions to the problem of storing hatching eggs, which can reduce the negative effects of storage and at the same time maintain the internal quality of the egg. One of these solutions is to spray the eggshell with oil before the storage process to close the pores as well as prevent the evaporation of water from inside the egg during the storage process (Hill and Hall et al., 1980). Another study suggests storing hatching eggs in rooms with high humidity to reduce the evaporation of water from inside the egg (Van den Brand et al., 2008).

(Reijrink et al., 2008) indicated that injecting hatching eggs with biological buffers to enhance the ability of egg albumen to regulate the pH value between 7.9 -8.4, during the storage period, which is the pH value appropriate for embryonic development.

2. Storage effect on the pH value of eggs albumen and yolks

The egg white (albumen) fills the utmost part of the egg contents. It constitutes 56-61% of the total egg weight, while the yolk, a protein colloidal solution, constitutes 27-32% (Sugino et al. 1997). When an egg is laid, the pH value of the albumen is 7.6, but after storing, changes occur in the composition of the white, including changes in the percentage of moisture, protein, and viscosity of the albumen, as well as in the pH value. As the yolk, when laying eggs, the pH value ranges 6.0 - 6.3, and then the pH value of the yolk slowly rises to reach a value ranging between 6.5 and 6.8, in addition to several other changes that may occur in the yolk during storage (Stern, 1991). Reijrink et al. (2008) suggest that the pH of the albumen is one of the most critical factors affecting the yolk index and the resistance of the vitelline membrane, as the albumen pH affects the chalaziferous layer, which is a layer consisting of fibers and a gelatinous substance covering the egg yolk, where the yolk membrane resistance depends on this layer's viscosity.

Nadia et al. (2012) demonstrated that the yolk acidity does not depend on bicarbonate, as is the case with egg albumen during storing eggs, but the reason for the slight rise in pH value of the yolk is due to the transfer of proteins as well as free amino acids from the albumen to the yolk through the yolk membrane which is due to the in osmotic pressure between the yolk and the white, and this transition continues until the point of equilibrium.

3. Biological Buffers

They are chemical solutions prepared in the laboratory and have the ability to resist considerable changes in the pH value in the medium in which the proteins are present when an acid or a base is added to them. The buffer solution regulates the pH value to be equal to or close to the fixed value. It consists of a mixture of a weak acid and its conjugate base (acid salt) or a weak base and its conjugate acid (base salt) (Hulanicki, 1987). Most of the biological solutions have been developed into solutions with effective protective properties called Good's buffers that are widely used in aqueous media and are insoluble in almost all organic solvents. Good's buffer solutions have some substantial properties, including they are non-toxic, soluble in water, chemically stable, slightly affected by changes in temperature and concentrations, they do not participate in biological reactions, and their absorption of ultraviolet rays is very low at a wavelength greater than 260 nm, as well as they are easy to prepare (Mohamed, 2016

4. The most important types of biological buffers among Good's buffer are:

4.1 HEPES buffer

[H; N-(2-Hydroxyethyl) piperazine-N′-(2-ethane sulfonic acid)] is another name for it. HEPES is one of the 20 types of Good's buffer, which is a buffer solution of sulfonic acid, commonly used in cell culture because it is the best in maintaining the pH value despite the changes occurring in the concentration of CO2 as a result of aerobic respiration compared to a bicarbonate buffer solution since HEPES has a pH range between 6.8 - 8.2 and a molecular weight of 238.30 Daltons (Taylor and Baicu, 2002). according to a study by Kannan et al (2017) to understand the effect of HEPES buffer on the biodegradation behavior of pure magnesium moiety in physiological solution. The results indicated that the HEPES buffer accelerates the decomposition of magnesium. When HEPES buffer at a concentration of 25 Mm was added to a physiological solution, it reduced the polarization resistance of magnesium by 98%, while increasing the HEPES buffer concentration to 50 Mm it increased the decomposition of magnesium

4.2 Tris buffer

T stands for Tris (hydroxymethyl)-amino methane in chemistry.. Tris is one of Good's buffers. Its chemical formula is C4H11NO3. It is widely used in biochemistry and molecular biology (Gomori, 1995). Tris buffer regulates the enzymes trypsin and chymotrypsin. It was found that it affects the covalent fixation in two ways in terms of reducing the number of Glyoxy1 groups available for support during covalent fixation (Sabrina et al. 2021). In a study, buffers were used to determine the molecular weight of some proteins and peptides compared to the electrophoresis system. The Tris buffer was among. When compared to other reagents, the Tris buffer gave more accurate results for the molecular weight range of proteins. This means that it can break down a large group of proteins into small peptides (Steven and Gregerson, 1986).

4.3 Bicine buffer

It is also called [B; N,N-Bis(2-hydroxyethyl)-glycine]. Bicine buffer is an organic compound, and it is one of the types of Good's buffers. Its chemical formula is C6H13NO4. It has a pH value between 7.6 - 9.0 (Lawson, 2003). The chemical composition of Bicine buffer is shown in Figure (1).

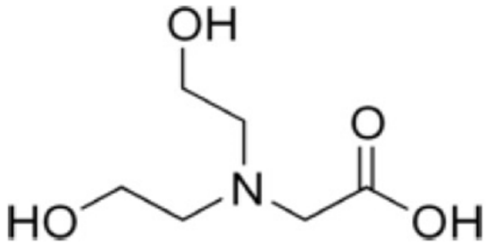

Figure 1. Chemical composition of Bicine buffer

4.4 Bis – Tris – Propane (BTP) buffer

It's known as [BTP; 1,3-Bis [Tris(hydroxymethyl)-methylamino] propane. It is one of the Good's buffers. It is a chemical substance used in buffer solutions as well as used in biochemistry and molecular biology. Its chemical name is C11H26N2O6. This buffer has some properties, including that is a white to yellow crystalline powder, highly soluble in water, its melting point ranging between 164-165 degrees Celsius, and it has a pH range between 6.3 - 9.5 (Song, 2003).

5. Effect of Bicine buffer injection on the pH value of albumen and yolks

Table 1 shows that the effect of storage did not differ significantly in the pH value of albumen and yolk on day zero of storage nor after 6 hours of injection and storage. Yet, at the end of the storage period, it was noticed that the treatment of eggs stored for 14 days was superior in the pH value of albumen and yolk to the other treatments (Muna,2021). This result is in line with the findings of many studies, which show that the longer an egg is stored, the higher the pH of the albumen, which affects the value of the yolk (Reijrink et al., 2008; Nadia et al., 2012).

Table (1) Effect of Bicine buffer injection on the pH value of whites and yolks

Transactions		Egg whites			Egg yolk		
stock	injection	zero	6 Hours	End stock	zero	6 Hours	End stock
		Effect of egg storage agent					
4		7.87	8.10	c 8.53	5.80	5.29	b 6.08
7		7.90	8.09	b 8.60	5.82	5.92	b 6.11
14		7.89	8.18	a 8.88	5.86	5.98	a 6.19
Storage probability		0.8033	0.0195	0.0001	0.2504	0.2234	0.0001
Injection factor strain							
Storage only		7.90	8.14	a 9.10	5.84	6.00	a 6.18
Egg hole only		7.88	8.13	b 8.77	5.84	5.92	b 6.09
Distilled water injection		7.90	8.14	a 9.00	5.84	5.93	a 6.18
Injection B50		7.88	8.06	c 8.28	5.80	5.92	ab 6.12
Injection B75		7.87	5.14	c 8.29	5.78	5.93	b 6.08
Possible injection		0.9438	0.3280	0.0001	0.5758	0.4590	0.0029
See the overlap between storage factor and injection factor							
4	Storage only	7.92	abc 8.20	b 8.98	5.78	ab 5.98	ab 6.14
	Egg hole only	7.86	c 8.02	c 8.08	5.84	ab 5.94	d 5.94
	Distilled water injection	7.84	abc 8.12	ab 9.10	5.72	b 5.92	a 6.22
	Injection B50	7.84	bc 8.08	c 8.28	5.78	b 5.88	ab 6.14
	Injection B75	7.92	bc 8.08	c 8.22	5.88	b 5.92	cd 5.98
7	Storage only	7.88	bc 8.08	b 9.00	5.86	b 5.90	ab 6.14
	Egg hole only	7.88	bc 8.10	b 8.92	5.82	b 5.88	ab 6.14
	Distilled water injection	7.90	abc 8.12	b 8.88	5.88	b 5.88	ab 6.14
	Injection B50	7.98	bc 8.08	c 8.22	5.80	ab 6.02	bc 6.08
	Injection B75	7.86	bc 8.10	c 8.28	5.74	b 5.92	bc 6.08
14	Storage only	7.92	abc 8.16	a 9.34	5.90	a 6.12	a 6.26
	Egg hole only	7.90	a 8.28	a 9.32	5.88	ab 5.94	ab 6.20
	Distilled water injection	7.98	abc 8.20	b 9.02	5.94	ab 6.00	ab 6.18
	Injection B50	7.84	c 8.04	c 8.34	5.84	b 5.88	ab 6.14
	Injection B75	7.84	ab 8.24	c 8.38	5.74	ab 5.96	ab 6.18
Possibility of interference		0.8618	0.0467	0.0001	0.2372	0.2516	0.0001
[2]General average		7.89	8.12	8.69	5.82	5.94	6.13
[3]SEM		0.0152	0.0151	0.0543	0.0152	0.0155	0.0130

1- Trial treatment - Storage only, Pocking only eggs with storage, Distilled water injection with storage, Circulated injection B_{50mM} with storage , Circulated injection B_{75mM} with storage. These treatments are the same for each period of egg storage, 4, 7 and 14 per day
2- The general average of an experiment.
3- SEM - standard error mean.
4- The different letters within a column indicate significant differences between the mean of treatment at.0.05 and 0,01.

(Muna,2021)

6. Effect of Tris buffer injection on the pH value of albumen and yolks

Table 2 shows the effect of Tris buffer injection on the pH value of the albumen and yolks of the hatching eggs stored for 4, 7, and 14 days. The table illustrates no significant differences in the effect of storage on the pH value of the albumen at day zero of storage, yet, after 6 hours of storage, the treatment of storing eggs for 14 days was superior over the rest of the treatments. At the end of the storage period, the treatment of eggs storage for 14 days was also superior to the other treatments. This is due to the increase in the water evaporation from inside the egg when the storage period is prolonged, leading to a loss of CO2 gas and thus a rise in the albumen pH value at the end of the storage period (Muna,2021). It is consistent with (Xavier et al., 2008), This result was reflected in the yolk pH value at the end of the storage period due to the transfer of some substances from albumen to yolk and vice versa through the yolk membrane, which led to an increase in the value of the yolk (Bakst and Holm, 2003).

Table (2) Effect of Tris buffer injection on the pH of egg white and yolk of hatching eggs stored for different periods

Transactions		Egg whites			Egg yolk		
stock	injection	zero	6 Hours	End stock	zero	6 Hours	End stock
		Effect of egg storage agent					
4		7.88	c 7.96	b 8.48	5.78	b 5.84	c 5.90
7		7.84	b 8.06	b 8.46	5.77	ab 5.86	b 5.99
14		7.84	a 8.18	a 8.78	5.82	a 5.93	a 6.12
Storage probability		0.3723	0.0001	0.0001	0.2314	0.0187	0.0001
Injection factor strain							
Storage only		7.86	8.08	a 8.99	5.93	a 5.83	a 6.16
Egg hole only		7.87	8.12	a 9.01	5.91	a 5.79	b 6.00
Distilled water injection		7.85	8.12	b 8.49	5.90	ab 5.81	b 5.99
Injection T50		7.85	8.01	c 8.21	5.85	ab 5.77	b 5.99
Injection T75		7.85	8.01	c 8.21	5.81	b 5.77	b 5.94
Possible injection		0.9934	0.0561	0.0001	0.5172	0.0416	0.0072
See the overlap between storage factor and injection factor							
4	Storage only	7.90	ab 8.10	c 8.88	5.76	bc 5.84	bcde 6.06
	Egg hole only	7.88	ab 8.06.	bc 8.96	5.78	ab 5.96	def 5.90
	Distilled water injection	7.84	bc 8.00	d 8.28	5.82	bc 5.80	def 5.90
	Injection T50	7.80	c 7.84	c 8.14	5.78	bc 5.82	f 5.82
	Injection T75	7.80	c 7.82	d 8.18	5.80	c 5.78	ef 5.86
7	Storage only	7.82	bc 7.98	c 8.84	5.86	bc 5.86	bcde 6.00
	Egg hole only	7.84	ab 8.10	c 8.92	5.72	bc 5.82	cdef 5.96
	Distilled water injection	7.90	ab 8.12	d 8.16	5.80	abc 5.94	bcd 6.04
	Injection T50	7.94	ab 8.06	d 8.20	5.76	bc 5.88	bcd 6.06
	Injection T75	7.92	ab 8.06	d 8.22	5.74	bc 5.84	def 5.90
14	Storage only	7.86	ab 8.16	a 9.24	5.86	a 6.08	a 6.30
	Egg hole only	7.88	a 8.20	ab 9.14	5.88	ab 5.96	ab 6.14
	Distilled water injection	7.82	a 8.24	bc 9.04	5.82	ab 5.96	bcd 6.04
	Injection T50	7.82	ab 8.14	d 8.30	5.78	ab 5.84	bc 6.10
	Injection T75	7.82	ab 8.16	d 8.22	5.78	bc 5.82	bcd 6.06
Possibility of interference		0.7820	0.0001	0.0001	0.4703	0.0053	0.0001
[2]General average		7.85	8.06	8.58	5.79	5.88	6.00

[3]SEM	0.0135	0.0192	0.0495	0.0117	0.0150	0.0186

1- Trial treatment - Storage only, Pocking only eggs with storage, Distilled water injection with storage, Circulated injection T_{50mM} with storage , Circulated injection T_{75mM} with storage. These treatments are the same for each period of egg storage, 4, 7 and 14 per day
2- The general average of an experiment.
3- SEM - standard error mean.
4- The different letters within a column indicate significant differences between the mean of treatment at.0.05 and 0,01.

(Muna,2021)

7. Conclusions

- The Bicine and Tris buffers used in the current study helped to maintain the pH of the albumen and yolk at the end of the storage period and made it within the allowed range for embryonic growth.
- We conclude from this study that the best buffer in terms of maintaining the pH value of the albumen and yolk is Bicine at the concentration of B75 and Tris at the concentration of T75.

References

[1] Bakst, M.R. ; and L. Holm,. 2003. Impact of egg storage on carbonic anhydrase activity during early embryogenesis in turkey. Poultry Science 82: 1193-1197 .

[2] Bakst. M. R. ; V. Akuffo ; D. Nicholson ;and N. French . 2012. Comparison of blastoderm traits from 2 lines of broilers before and after egg storage and incubation . Poultry Science 91 :2645–2648 .

[3] Dymond, J. ; B. Vinyard ; A.D.Nicholson ; N.A.French ; M.R. Bakst ; .2013. Short periods of incubation during egg storage increase hatchability and chick quality in long-stored broiler eggs. Poultry Science 2013 Nov; 92(11):2977-87.

[4] Fasenko,G.M.2007. Egg Storage and the Embryo. Poultry Science. 86:1020–1024 .

[5] Gomori, G.1955. Preparation of Buffers for Use in Enzyme Studies. Methods Enzymology. 1, 138-146 .

[6] Hill, A. T. ; and J. W. Hall. 1980. Effects of various combinations of oil spraying, washing, sanitizing, storage time, strain, and age of layer upon albumen quality changes in storage and minimum sample sizes required for their measurement. Poultry Science. 59:2237–2242.

[7] Hulanicki, A.1987. Reactions of acids and bases in analytical chemistry. Horwood. ISBN 0853123306.

[8] Kannan M. B. ; H. Khakbaz ; and A. Yamamoto. 2017 . Understanding the influence of HEPES buffer concentration on the biodegradation of pure magnesium: An electrochemical study. Materials Chemistry and Physics . 47-56.

[9] Lawson , G. .2003. Amine Plant Corrosion Reduced by Removal of Bicine.

[10] Meijerhof, R. 1992. Pre-incubation holding of hatching eggs. World's Poultry Science. J. 48:57–68.

[11] Mohamed T. ; and A.P.Coutinho . 2016. Organic-phase biological buffers for biochemical and biological research in organic media. Molecular Liquids. 197-205 .

[12] Muna A. H. AL- Fahdawi.2021. Injecting Hatching Eggs before storage with Different Biological Buffers and Effect on Some Hatching Standards and Specifici of Chick Characteristics. Ph. D. Dissertation, College of Agriculture, University of Al- Anbar, Iraq.

[13] Nadia, N. A.A. ; S.R.Z.Bushra ;A.F. Layla ;M.A. Fira. 2012. Effect of coating materials (gelatin) and storage time on internal quality of chicken and quail eggs under refrigeration storage Poultry Science, 32 (1) (pp. 107–115.

[14] Reijrink, I.A.M. ; Meijerhof, R. ; B.Kemp; and B.R.Vander. 2008. The chicken embryo and its micro environment during egg storage and early incubation. World's Poultry Science Association 64:581-598.

[15] SabrinaA.B.; R.MorellonSterling. ; D.deAndrades. ; R.C.Rodrigues. ; E. Siar; A. Aksas; J. Pedroche; M. D. Millán and R. Fernandez-Lafuente. 2021. Effect of Tris Buffer in the Intensity of the Multipoint Covalent Immobilization of Enzymes in GlyoxylAgaroseBeads.AppliedBiochemistryandBiotechnology volume 193, pages2843–2857.

[16] Song, L. 2003. Detection of farnesyl diphosphate accumulation in yeast ERG9 mutants. Anal. Biochem. 317 (2): 180–185.

[17] Stern, C. D. 1991. The sub-embryonic fluid of the domestic fowl and its relationship to the early development of the embryo. Pages 81-90 in Avian Incubation. G. Tullet, ed. Butterworth-Heinemann, London, UK.

[18] Steven P. F . ; and D. S. Gregerson . 1986 . Peptide and protein molecular weight determination by electrophoresis using a high-molarity tris buffer system without urea . Analytical Biochemistry. 83-88.

[19] Sugino H. ; T. Nitoda ; L.R.Juneja ; .1997. General chemical compositio of hen eggs. In: Yamamoto T, Juneja LR, HattaH,KimM(eds) Hen Eggs: Their Basic and Applied Science. Boca Raton, FL: CRC Press, pp. 13–23.

[20] Van den Brand, H. ; I. A. M. Reijrink; L. A. Hoekstra; and B. Kemp. 2008. Storage of eggs in water affects internal egg quality, embryonic development, and hatchling quality. Poultry Science 87:2350– 2357.

[21] Xavier, I.M.C. ; S.V.Cancado ; T.C.Figueiredo; L.G.C.Lara; A.M.Q.Lana ; M.R.Souza ;and N.C.Baiao; . 2008. Qualidade de ovos de consumo submetidos a diferentes condições de armazenamento. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 60: 953-959.