THE EFFECT OF EGG COATING ON BROILER BREEDER EGGS DURING LONG
TERM STORAGE

by

JAVIER A. SANTOS

(Under the Direction of Joseph Mauldin)

ABSTRACT

Three different polyvynil alcohol formulas were used in this study to test the effects of egg coating during egg storage conditions. Broiler breeder eggs were coated and stored for 4 different time periods (1 day, 1 week, 2 weeks and 3 weeks). Samples from each treatment group were analyzed for internal egg quality. The Haugh units and albumen pH showed some significant differences between the coated and uncoated eggs even after only one day of storage. The hatch of fertiles for coated groups showed improvement over the uncoated groups, but the flock performance affected the results significantly. Egg coating still holds potential for industrial application, but the effects of flock's age on the effectiveness of egg coating have to be studied further.

INDEX WORDS: egg coating, polyvynil alcohol, hatchability, Haugh units, albumen pH, egg storage

THE EFFECT OF EGG COATING ON BROILER BREEDER EGGS DURING LONG ${\bf TERM\ STORAGE}$

by

JAVIER A. SANTOS

BSA, University of Georgia, 2008

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

Master of Science

ATHENS, GEORGIA

2011

© 2011

Javier A. Santos

All Rights Reserved

THE EFFECT OF EGG COATING ON BROILER BREEDER EGGS DURING LONG TERM STORAGE

by

JAVIER A. SANTOS

Major Professor: Dr. Joseph Mauldin

Committee: Dr. Joseph Mauldin

Dr. Adam Davis Dr. Jeff Buhr

Electronic Version Approved:

Maureen Grasso Dean of the Graduate School The University of Georgia August 2011

TABLE OF CONTENTS

		Page
LIST OF	TABLES	v
LIST OF	FIGURES	vi
СНАРТЕ	ER	
1	Introduction	1
2	Literature Review	3
	Egg storage	3
	Egg coating	4
	Previous works with PVA	5
3	Materials and Methods	6
	Preparation	6
	Experimental Design	7
	Storage conditions and set times	7
	Egg Quality studies	8
	Incubation and Hatching	9
4	Results and Discussion	10
	Internal egg quality	10
	Hatchability	13
5	Conclusion	15
REFERE	ENCES	17

LIST OF TABLES

	Page
Table 1: Results from scouting trial (Mauldin Buhr 2008)	25
Table 2: pH Measurements by Treatment and Storage time	25
Table 3: Haugh units by Treatment and Storage time	25
Table 4: Shell Strength Force (g) by Treatment and Storage time	25
Table 5: Vitelline Membrane Force (g) by Treatment and Storage time	26
Table 6: Means by treatment for fresh eggs	26
Table 7: Means by treatment for 1 week of storage	26
Table 8: Means by treatment for 2 week of storage	26
Table 9: Means by treatment for 3 week of storage	27

LIST OF FIGURES

	Page
Figure 1: pH Measurements by Treatment and Storage time	20
Figure 2: Haugh units by Treatment and Storage time	20
Figure 3: Percent (%) Yolk Moisture and Yolk Solids for Fresh eggs	21
Figure 4: Percent (%) Yolk Moisture and Yolk Solids for 1 wk	22
Figure 5: Percent (%) Yolk Moisture and Yolk Solids for 2 Wks	23
Figure 6: Percent (%) Yolk Moisture and Yolk Solids for 3 Wks	24

CHAPTER 1

Introduction

The poultry industry is often considered a role model for the industrialization of agriculture. The poultry industry is a vertically integrated production, processing, and distribution system where the physical production of birds is handled almost entirely by contract growers. Unfortunately, the integrated poultry industry is occasionally forced to store their eggs for long periods of time. Long term storage is known to have a detrimental effect in hatchability. Eggs that are no longer able to hatch are set in incubators, resulting in an economical loss for the company.

Egg coating has drawn the attention of many people in the hatchery management field. A spray on material that can preserve the micro environment of an egg during storage for weeks does sound appealing to many hatcheries. After some preliminary testing, polyvinyl alcohol stood out as the ideal candidate for an egg coating solution.

Companies like International Poultry Breeders (I.P.B.) ship eggs to many places around the world. These eggs can take up to 2 weeks to get set in an incubator. It is known that hatchability tends to dramatically decrease after 7 days of storage. IPB has seen drops of 7-8% in hatchability of their batches. If companies like IPB had a method of preserving the quality of their eggs throughout storage time, it would provide a tremendous bonus in increased revenue.

The safety, quality and shelf life of eggs is dependant on CO2 content. Carbon dioxide starts to escape the egg resulting in an increase of egg albumin pH. (K.M. Keener, 2001) Researchers have found that embryos require an albumin pH of 8.2 for optimum development. (A.M. Reijrink, 2010). Providing a water soluble coating that can reduce the gas exchange rate during storage could potentially improve hatchability. The main goal of this project is to increase the hatchability of long term storage eggs using a polymer coating.

CHAPTER 2

Literature Review

Egg storage

Egg storage has been a problem for the poultry industry for quite some time. The current industry standards for egg storage include: 55°F, 85% RH and the 90° turning daily. Hatchability of the eggs will suffer if any of these guidelines are not met. Even in these conditions, hatchability is known to decrease after 7 days of storage. Studies like A.M. Reijrink et al (2009) confirm that storing eggs for longer than 7 days drastically affects hatchability. It is not yet clear what causes embryos to die after one week of storage.

Yassin et al (2008) proposes that the age of the breeder flock plays a factor in the durability of the embryos during the storage phase. In their experiment, the eggs from young breeder flocks (25 to 30 wks of age) showed a greater decrease in hatchability after 8 to 14 days of storage than the eggs of older breeder flocks (51 to 60 wks of age). However, Meijerhof et al. (1994) and Elibol et al (2002) have reported in their work that older breeder flock eggs (59 wks and 53 wks respectively) suffer a greater hatchability loss after prolonged storage when compared to younger breeder flock eggs (37 wks and 30 wks respectively). There is no clear idea why eggs from different sizes and thickness would give such various results, but the one constant that remains is the decline in hatchability over time of storage.

Egg coating

Egg coating has been used in many studies involving shelf life extension.

Panuwat Suppakul et al (2010) used a cellulose based coating to preserve egg quality over 28 days of storage. The coated egg quality decreased after 7 days from grade AA to grade A, and they stayed at that level for the rest of the 4 weeks. Uncoated egg quality decreased from grade AA to grade A in only 5 days, and continually deteriorated to grade B in the following 4 weeks.

Chitosan-based coating has also been used for increasing shelf life for table eggs. Caner C. and Cansiz O. (2007) found that Chitosan-based coating containing lactic acids and propionic acids could maintain the quality of eggs in grade A after being stored for 4 weeks. Other types of chitosan-based coatings have also been seen to increase shell strength (Caner C. and Cansiz O. 2008).

The usefulness of egg coating in fertilized egg storage remains to be tested. The way coating preserves table eggs during long terms of storage could also be applied to fertilized eggs.

Previous works with Polyvynil alcohol coating (PVA)

Dr. Joseph Mauldin and Dr. Jeff Buhr from the University of Georgia performed some scouting trials for Dupont in search for a proper egg coating material that would preserve eggs for storage. The results can be seen in **Table 1**. During these trials, 11 formulas were tested with 3 week storage and fresh eggs. The formulas included different variations (dyes) and concentration levels of polyvinyl alcohol. The method used for applying the coating was egg dipping. The eggs were stored at 68°F. Some of the groups had the coating removed while others were incubated with the coating still on (specified by Rinsed or Not Rinsed). Egg candling took place at day 14 of incubation to check for fertility.

The results varied drastically depending on the formula. The data also showed that eggs with coating during incubation had really low hatchability or didn't hatch at all. The top performers of this trial included 6% and 9% PVA with blue dye (2 and 3). These formulas showed a 100% increase in hatchability for groups with 3 weeks of storage when compared to the control. These were the formulas that were selected for the following experiment.

CHAPTER 3

Materials and Methods

Preparation

A modified SurePip mechanical hatching egg sanitizer was modified to apply the coating material. The machine was specifically selected for this procedure due to its precise temperature control and multiple high pressure spray nozzles, a feature that ensured that the coating material was applied in its liquid form. The PVA solution had to be kept at a temperature of 95°F before application to prevent it from clogging the nozzles; at room temperature the formula solidifies and gains its coating properties. The SurePip mechanical egg sanitizer is divided in 2 zones; there is the sanitation zone and the disinfectant zone. Each of these zones has its own liquid feeding line. A row of nozzles was added to the lower section of the disinfectant application zone. The modification ensured that the machine could cover the eggs completely in all zones.

The scouting trials showed that the coating had to be removed before the eggs are placed in the incubator. Not doing so would prevent most of the eggs from hatching. The SurePip machine was used to rinse off the coating material after the storage time was complete. A cycle of hot water was enough to remove the PVA coating.

Eggs were purchased from Harrison Poultry Company. The flock was a 38 weeks old Heritage x Heritage with good fertility and hatchability numbers. There were 196 randomly picked eggs assigned to each treatment group.

Experimental design

The experiment included 4 coating treatments and 4 storage times, making it a total of 16 treatment groups. Treatments were: control (non-coated), 6 % PVA formula, 9 % PVA formula, and 9 % formula following egg sanitization. The last group was included to determine the effect of using a chloride based sanitizer on the eggs before applying the egg coating. The eggs were first passed through a cycle of chlorine based sanitizer and coated quickly afterwards. The four storage times were 1 day (fresh), 1, 2, and 3 weeks.

Storage conditions and set times

For the main trial it was planned to store the eggs as is normal for long storage in the poultry industry. The egg cooler was calibrated at 58° F and 85 % R. H. The eggs were turned a 90° degree angle once per day during storage. It was decided that the best procedure would be to set all the eggs at once so that the data would not be influenced by different weeks of setting. The eggs that were to be stored for 3 weeks were collected, coated and placed in storage. One week later, the eggs that were to be stored for 2 weeks were collected coated and placed in storage. After two more weeks, all of the eggs were collected and treated following the above protocol.

Egg Quality Studies

Twelve eggs were randomly picked from all of the treatment and control groups to be used a sample for the egg quality studies. Each individual egg provided data for the following tests:

Haugh units and Shell strength – Each egg was weighed on the Haugh unit (HA) balance, then shell strength tested on the texture analyzer (TA). Once the egg was cracked, it was laid in on a flat surface where the albumen height was measured. HA were calculated by the computer using both egg weight and albumen height. The albumen was separated from the yolk and put in a properly labeled whirlpak bag and stored in the refrigerator (4°C).

Vitelline membrane (VM) strength - The yolk was moved into an empty petri dish (100 x 15) and placed on the TA. The machine was manually stopped when the vitelline membrane ruptured and released the yolk material. The yolk was then transferred into a properly labeled whirlpak bag and stored in the refrigerator (4° C)

Parameters for Texture Analyzer Shell Strength and Vitelline Membrane

Project	Load	Test	Distance	Trigger	Trigger
	Cell	speed		force	distance
Shell	5kg	2mm/sec	1mm	1g	2mm
Disc VM auto	1kg	1mm/sec	11.5mm	5g	2mm

^{*}A 3" diameter compression platen was used for both vitelline membrane and shell strength testing.

Albumen pH – The albumen samples were taken out of the refrigerator to equilibrate to room temperature. The samples were stomached for 1 minute at normal speed using a Stomacher 80 blender. The pH for each sample was recorded using an Orion 525Aplus meter.

Yolk moisture – 5 grams of homogenized sample for each egg were paced in individual aluminum drying pans. The pans were placed in a 100oC forced air drying oven for 18-24 hours. The samples were then allowed to equilibrate in desiccators for at least an hour before weighing.

Incubation and Hatching

Three UGA NatureForm incubators were used for the incubation. The eggs were candled at 14 days of incubation to remove unfertilized eggs and improve % hatch of fertiles. After 21 days, the birds were checked for hatchability and hatch of fertiles.

CHAPTER 4

Results and Discussion

Internal egg quality

The effects of treatment and storage time on internal egg quality appeared to be more dramatic than the hatchability numbers. Higher albumen pH was recorded for the non-coated control eggs than the coated groups for all four storage time periods (**Figure 1**). The effect of the coating was immediate, considering that even the fresh eggs showed significant differences between the treatments and the control (P < 0.05). As shown in **Table 2**, the treatment group with 9% PVA w/sanitizer had the lowest pH. Even the other experimental groups (6% and 9% PVA) showed a significant difference from the control. Significant differences between the two formulas were only seen after 3 weeks of storage, with 9% PVA maintaining a lower pH value than the 6% PVA.

As seen in **Figure 1**, the albumen pH of an egg will increase due to the escaping of CO₂ gas through the egg shell pores (Stadelman, W.J. 1995). After one week of storage, the control group reached a higher pH "plateau" which was around 9.2. The coating reduced the amount of CO₂ that is released by the egg during the storage time, which resulted in a significantly lower pH value. Reasons behind the success of applying chloride sanitizer before the egg coating remain unknown.

Another measure of egg quality, the Haugh units (HU), didn't show the variation seen with the albumen pH (Table 3). Considering that albumen pH and Haugh units of an egg are closely correlated (Li-Chan, et al. 1995), we were expecting to find a trend similar to that of the albumen pH. Figure 2 presents that only eggs stored for more than one week showed significant differences between the treatments. The trend previously seen with albumen pH is not found in Haugh units. Some of the treatment groups don't manage to statistically differentiate from the control. The 9% PVA w/sanitizer, which excelled in maintaining a lower albumen pH, was not significantly different from the control after one week of storage. It took 2-3 weeks of storage for the 9% PVA w/sanitizer to show a significantly higher HU from the control. The HU values for the 6% PVA and 9% PVA formulas were not consistent compared to the control. Both treatment groups had a significantly higher HU value after one week of storage, but quickly fell after the second week of storage.

The shell strength force (SSF) recorded in this experiment didn't show any significant differences between the treatment groups with the exception of the eggs stored for 2 weeks (**Table 4**). The eggs from the 6% PVA coating required a significantly higher force application before cracking than the control group. Random differences among the control and treatment groups could be related to the small sample size selected for the egg quality studies.

Similar to the results for SSF, the data for the vitelline membrane force (VMF) showed no significant differences among the treatment groups and the control (**Table 5**). The 9% PVA w/sanitizer treatment did require a significantly higher application of force to break, but the lack of an obvious relation between VMF and the increase of storage time makes it hard to deduce what it means. It is very likely that the VMF data is being affected by the small sample size.

The results for percent yolk moisture can be seen in **Figures 3-6**. Other studies have shown that the yolk moisture of a table egg will increase over time in storage conditions. Its counterpart, % of yolk solids will decrease (D. R. Jones, 2007 and W. Guo et al. 2007). W. Guo points out that albumen moisture is lost overtime due to a migration of water from the albumen into the yolk, which results in the increase of moisture content found in the yolk. The coating material appears to have no effect in this process.

Although, there are some significant differences among the treatment groups and the control groups after one week of storage (**Figures 4-6**), the variation is not consistent with the progress of time. In weeks 1 and 3, none of the coated groups are significantly different from the control. Only in week 2, the 6% PVA and the 9% PVA w/sanitizer showed a significant difference from the control. These random variations could be attributed to the relatively small sample size picked for this part of the experiment.

Hatchability

The results for hatchability and hatch of fertiles are presented in **Tables 6-9**. Only hatch of fertiles (HOF) were statistically analyzed, since it avoids being mislead by the variation caused by infertility. Changes in the % HOF should be more closely related to egg coating than % hatch. The% HOF means for the fresh eggs showed significant differences between the treated groups and controls. The 6 % PVA was not significantly different from either the controls or the 9% PVA, but the other two treatment groups (9% PVA and 9% PVA w/san) did manage to significantly improve % HOF compared to the comtrol. These were eggs that were coated and stored for only 24hrs. The immediate effect in hatchability mirrors that of the albumen pH.

Unexpected results were seen after one week of storage (**Table 7**). All of the groups had similar hatching numbers to the fresh eggs after being stored for one week. Storage conditions were properly managed, but it doesn't explain why the birds were able to hatch as if the eggs were just laid. There were no significant differences found between the treatment groups and the control for this storage time batch. The 9% PVA and 9% PVA w/san had the highest % HOF, but there were not significantly different from the 6% PVA and the control.

The storage time started to show its detrimental effects after 2 weeks of storage (**Table 8**). The % HOF for the control group dropped ~8.5%. The 9% PVA was not significantly different from the control with only ~1.0% of improvement in HOF. The other two treatments did show a significantly greater improvement in % HOF. The 6 % PVA even kept the hatch of fertiles above 90%. The % HOF is still higher than expected for all groups.

Hatch of fertiles after 3 weeks was higher than expected. Preliminary trials done by Dr. Mauldin and Dr. Buhr had shown hatchability numbers way below 50%, while some of the coated groups had maintained the % hatchability around 60% (**Table 1**). The results after 3 weeks of storage show a 69.97% HOF with and overall 68.66% hatchability. Two of the egg coating treatments (9% PVA and 9% PVA w/san) were actually ~2.0% HOF below the control. Only the 6% PVA showed a slight improvement over the control group having 75.34% HOF. These results raised some question regarding the flock selected for this experiment. The 38 week old flock provided eggs that had a 68-70% HOF after the eggs were stored for 3 weeks. Most flocks would be closer to 40% HOF after 3 weeks of storage.

CHAPTER 5

Conclusion

Egg coating has been approached by many scientists as an alternative to extend shelf-life of table eggs, but the scouting trials done by Dr. Mauldin and Dr. Buhr showed that a polyvynil alcohol coating had the potential to be used in other areas of the industry. The percentage HOF in some of the PVA coated groups essentially doubled the performance of the non-coated controls. Similar results were not present in this experiment. The lack of consistency in the results could be attributed to the selected flock. The 38 week old Heritage x Heritage performed incredibly well during this experiment. Even after the eggs were stored for three weeks the % HOF was near 70%, a number that is expected after only 14 days of storage. Having such a high performing control group makes it difficult to improve the numbers using the PVA coating. The coated groups could be improving the hatchability, but the good performance by the flock made the differences between the groups appear insignificant. A second trial has to be done to test the effects of flock age and performance on the effectiveness of the coating.

Regardless of the exceptional flock used, the egg coating treatments still managed to show some improvement in % HOF. The fresh eggs, only coated for 24 hours, showed a significant difference between the treatment groups and the control. In every storage time egg batch, the coated groups always managed to have a higher performance than the control.

The egg quality studies were done to provide information about the changes that could occur internally due to the coating material. The relatively small sample size made it difficult to relate the internal composition of the egg to the hatching capacity. The increase in yolk moisture during the storage period occurred as expected, and it appears that coating the egg doesn't have consequences in the natural process. The 9% PVA w/san treatment did show a significantly lower albumen pH accompanied by a high Haugh units value, but good internal quality didn't directly translate to better hatchability. Egg quality is still not an absolute determining factor for success in hatchability for broiler breeder eggs.

The scouting trials performed by Dr. Mauldin and Dr. Buhr showed that the benefit of coating eggs was apparent. The treatment offered potential to the hatching egg industry to increase hatchability performance. The main trials of this experiment were not able to come near the expectations sparked by the previous experiments. Egg coating still holds some great potential for the hatching egg industry. Perhaps it can help store grandparent flocks breeder eggs for longer periods of time or improve the hatchability of eggs that have to be shipped across the ocean. Further investigation using different types of coating would provide useful information regarding the preservation of fertilized eggs during storage.

REFERENCES

A. M. Reijrink, D. Berghmans, R. Meijerhof, B. Kemp, and H. van den Brand, 2010. Influence of egg storage time and preincubation warming profile on embryonic development, hatchability, and chick quality. Poultry Science 89: 1225-1238.

A.M. Reijrink, R. Meijerhof, et al. 2008. The chicken embryo and its micro environment during egg storage and early incubation. World's Poultry Science Journal Vol. 64 Issue 4 pp 477-610

Caner C., Cansiz Ö., 2007. Effectiveness of chitosan-based coating in improving shelf-life of eggs. Journal of the science of food and agriculture. v. 87, no. 2, p. 227-232

Caner C., Cansiz Ö., 2008. Chitosan coating minimizes eggshell breakage and improves egg quality. Journal of the science of food and agriculture. v. 88, no. 1, p. 56-61

D. R. Jones. 2007. Egg Functionality and Quality During Long-Term Storage. International Journal of Poultry Science 6 (3): 157-162.

K.M. Keener, J.D. LaCrosse, J.K. Babson, 2001. Chemical Method for Determination of Carbon Dioxide in Egg Yolk and Egg Ablumen. Poultry Science Association

Meijerhof, R., J. P. T. M. Noordhuizen, and F. R. Leenstra, 1994. Influence of preincubation treatment on hatching results of broiler breeder eggs produced at 37 and 59 weeks of age. Br. Poult. Sci. 35:249-257

Li-Chan, E. C., W. D. Powrie, and S. Nakai. 1995. The chemistry of eggs and egg products. Egg Science and Technology. 4th ed. pp105-175 W. J. Stadelman and O. J. Cotterill, ed. Food Products Press, New York, N.Y.

O. Elibol, S.D. Peak and J. Brake, 2002. Effect of Flock Age, Length of Egg Storage, and Frequency of Turning During Storage on Hatchability of Broiler Hatching Eggs. Poultry Science 81: 945-950.

Panuwat Suppakul, Kowit Jutakorn, Yannawit Bangchokedee, 2009. Efficacy of cellulose-based coating on enhancing the shelf life of fresh eggs. Journal of Food Engineering 98: 207-213.

Stadelman, W. J. 1995. The preservation of quality in shell eggs. Egg Science and Technology. 4th ed. pp 67-79 W. J. Stadelman and O. J. Cotterill, ed. Food Products Press, New York, N.Y.

Yassin, H., A. G. J. Velthuis, M. Boerjan, J. van Riel, and R. B. M. Huirne, 2008. Field study on broiler eggs hatchability. Poultry Science 87:2408-2417

W. Guo, S. Trabelsi, S. O. Nelson, nd D. R. Jones. 2007. Storage Effects on DiaelectricProperties of Eggs from 10 to 1800MHz. Journal of Food Science v. 72, Nr. 5, E335-E340

Figure 1.



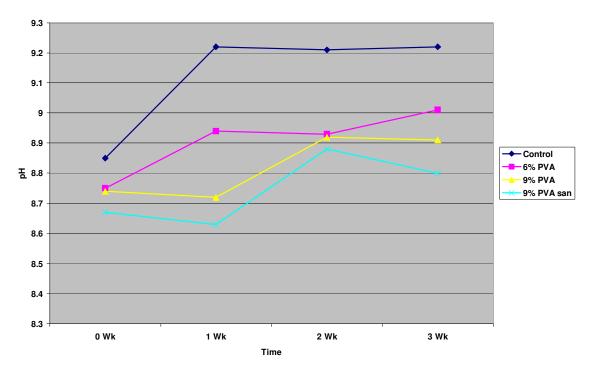


Figure 2.

Haugh units by Treatment and Storage time

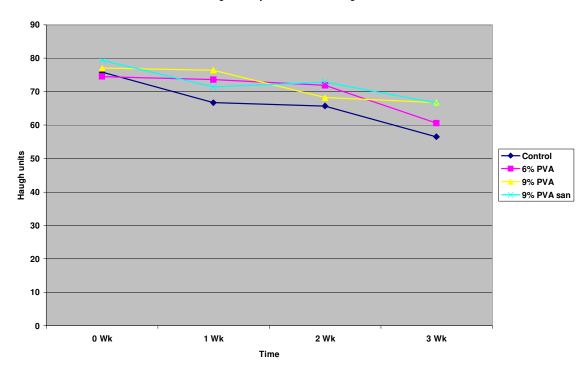


Figure 3.

% Yolk Moisture and % Yolk Solids for fresh eggs

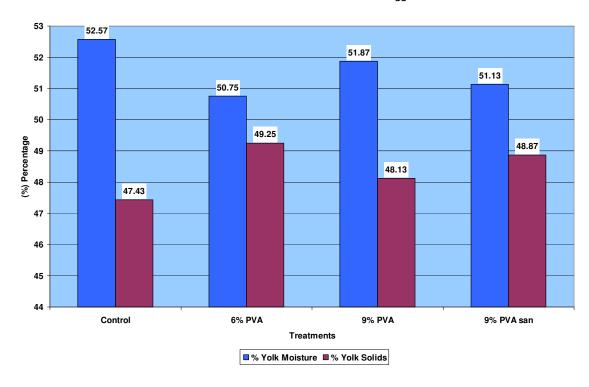
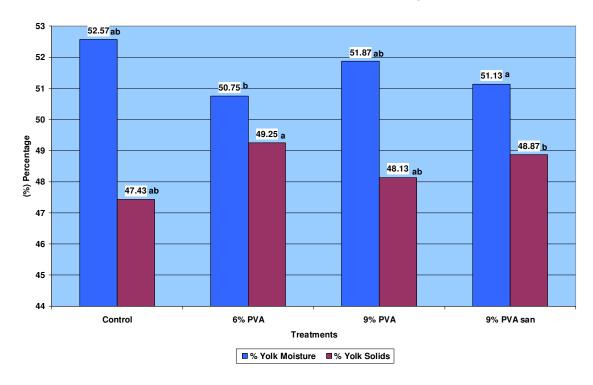


Figure 4.

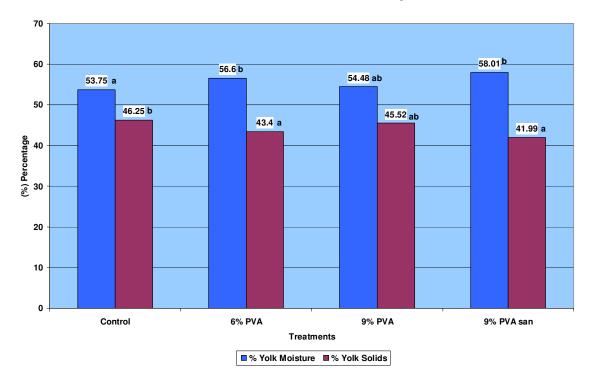
$\%\,\mbox{Yolk}$ Moisture and $\%\,\mbox{Yolk}$ Solids for $\,$ 1 Wk of storage



 $^{^{\}mathrm{a-b}}$ means with different superscripts differ significantly (P < 0.05)

Figure 5.

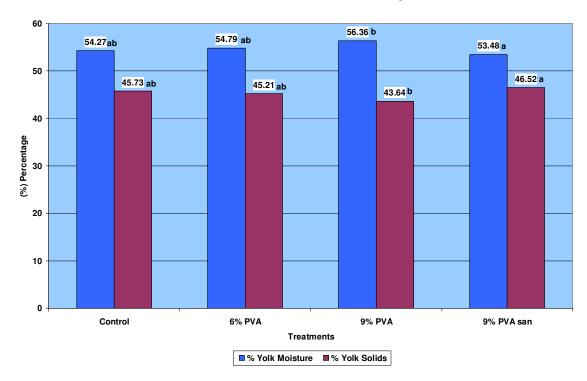




 $^{^{\}mathrm{a-b}}$ means with different superscripts differ significantly (P < 0.05)

Figure 6.

% Yolk Moisture and % Yolk Solids for 3 Wks of storage



 $^{^{\}mathrm{a-b}}$ means with different superscripts differ significantly (P < 0.05)

Table 1. Results from scouting trial (Mauldin Buhr 2008)

Formula Group	Rinsed/(R) or Not Rinsed (NR)	# Eggs	# Chicks	% Fertility	% Hatch of Fertiles	% Hatch
0 Control		120	40	95.83	34.78	33.33
2	R	87	53	95.40	63.86	60.92
3	R	72	45	98.61	66.20	62.50
4	R	28	10	96.43	37.03	35.71
5	R	30	20	96.67	68.97	66.67
6	NR	30	1	83.33	4.00	3.33
7	R	30	4	96.67	13.79	13.33
9	NR	30	0	96.67	0	0
10	NR	28	9	96.43	33.33	32.14
11	NR	30	15	88.99	56.19	50.00

Table 2. pH Measurements by Treatment and Storage time

	0 Wk	1 Wk	2 Wk	3 Wk
Control	8.85 ^c	9.22 ^c	9.21 ^b	9.22 ^d
6% PVA	8.75 ^b	8.94 ^b	8.93 ^a	9.01 ^c
9% PVA	8.74a ^b	8.72 ^b	8.92 ^a	8.91 ^b
9% PVA				
san	8.67 ^a	8.63 ^a	8.88 ^a	8.8 ^a

a-d means with different superscripts differ significantly (P < 0.05)

Table 3. Haugh units by Treatment and Storage time

	0 Wk	1 Wk	2 Wk	3 Wk
Control	75.83	66.62 ^a	65.64 ^a	56.45 ^a
6% PVA	74.44	73.56 ^b	71.86 ^b	60.6 ^{ab}
9% PVA	76.98	76.39 ^b	68.22 ^{ab}	66.71 ^b
9% PVA				
san	79.39	71.42 ^{ab}	72.88 ^b	66.54 ^b

^{a-b} means with different superscripts differ significantly (P < 0.05)

Table 4. Shell Strength Force (g) by Treatment and Storage time

Control	4181	3760	4279°	4312
6% PVA	4119	4442	3861 ^a	4342
9% PVA	4185	4314	4031 ^{ab}	4131
9% PVA san	4016	4418	4265 ^{bc}	3861

^{a-c} means with different superscripts differ significantly (P < 0.05)

Table 5. Vitelline Membrane Force (g) by Treatment and Storage time

		(C)		
	0 Wk	1 Wk	2 Wk	3 Wk
Control	129.3	112.5	96.1	85 ^a
6% PVA	109.9	93.5	88.4	83.7 ^a
9% PVA	134.4	93.4	99.4	75.5 ^a
9% PVA san	120.5	82.2	84.9	110.9 ^b

a-b means with different superscripts differ significantly (P < 0.05)

Table 6. Means by treatment for Fresh eggs

Treatment	Hatchability %	Hatch of Fertiles %
Control	86.89	89.20a
6 % PVA	89.49	91.32ab
9 % PVA	92.82	94.18ab
9 % PVA San	94.59	96.21b

^{a-b} means with different superscripts differ significantly (P < 0.05)

Table 7. Means by treatment for 1 week of storage

Treatment	Hatchability %	Hatch of Fertiles %
Control	92.00	93.30
6 % PVA	86.48	88.21
9 % PVA	93.33	95.22
9 % PVA San	91.48	94.16

Table 8. Means by treatment for 2 weeks of storage

Treatment	Hatchability %	Hatch of Fertiles %
Control	84.80	85.74a
6 % PVA	88.81	91.44b
9 % PVA	81.82	86.86a
9 % PVA San	86.65	89.95b

^{a-b} means with different superscripts differ significantly (P < 0.05)

Table 9. Means by treatment for 3 weeks of storage

Treatment	Hatchability %	Hatch of Fertiles %
Control	68.66	69.97
6 % PVA	71.01	75.34
9 % PVA	66.56	71.94
9 % PVA San	66.08	72.09