The Role of Magnesium and Calcium in Eggshell Formation in Tsaiya Ducks and Leghorn Hens**

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ABSTRACT : Tsaiya ducks and Leghorn hens are the two major laying birds raised in Taiwan. They are all excellent egg layers. Tsaiya ducks are small in body size (1.3 kg) with bigger egg weight (65 g) and stronger eggshell breaking strength than eggs from hens. The eggshell consists mainly of calcium carbonate, hence calcium plays an important role in the eggshell formation. Magnesium is also present in eggshell in small amounts, which may have effect on maintaining eggshell quality. In comparison studies, it was shown that the duck eggshells contained higher calcium and lower magnesium content than chicken eggshells. The eggshell magnesium content was not affected by the dietary magnesium levels (690-2380 ppm) in ducks, but in hens, it increased linearly with dietary magnesium levels. The palisade layer (5000×) of the eggshell was found to have a compact form for ducks while there are many hallow vesicles in chicken eggshells. The eggshell magnesium deposition model is different for ducks and hens with ducks having a one-peak and hens having a two-peak model. The calcium deposition model is similar for both birds. Both the carbonic anhydrase specific activity and total activity in the shell gland mucosa of ducks are higher than those in hens. Ducks retain higher magnesium and lower calcium in the shell gland mucosa and secret less magnesium and more calcium into the shell gland lumen for eggshell deposition. The ATPase specific activity is maintained fairly constant during the eggshell forming stage, indicating continuous calcium transport into the shell gland lumen for eggshell formation. The magnesium content in duck eggshells is much lower than that in hens indicating that the magnesium content in the eggshell may have an effect on eggshell quality. ***(Asian-Aust. J. Anim. Sci. 2003. Vol. 16, No. 2 : 290-296)***

Key Words : Calcium, Magnesium, Eggshell, Tsaiya Duck, Leghorn Hen

INTRODUCTION

Leghorn hens and Tsaiya ducks are the two major egg laying fowls raised in Taiwan. They play an important role in Taiwan's poultry industry. As reported in the Agricultural Statistics Yearbook (2000), the numbers of Leghorn hen and Tsaiya duck raised in Taiwan are 35,375,000 and 2,921,000, respectively, with an annual egg production of 7,270 million eggs for hens and 478 million eggs for ducks. The duck eggs are produced mainly for making processed eggs such as Pitan (alkalized egg) or salted eggs for human consumption.

The laying ducks raised in Taiwan are called Tsaiya ducks or domestic ducks (*Anas platyrhynchos var. domestica*). In general, they are called brown Tsaiya ducks. Tsaiya ducks are good egg producers with a small body size (1.2-1.4 kg) and lay eggs (16 wk) earlier than white Leghorn hens. Duck eggs are normally larger with a thicker eggshell and higher egg breaking strength than chicken eggs. In our studies on the utilization of calcium between laying Tsaiya ducks and Leghorn hens (Chen and Shen, 1989), we found that the magnesium content (0.13%) in the eggshell was lower in duck eggs than in chicken eggs (0.41%) irrespective of the dietary calcium levels. Hence we investigated why there was lower magnesium content in duck eggshells? Was there a correlation between the magnesium content and eggshell strength? It was worthwhile to design experiments to determine the difference in calcium and magnesium utilization in the eggshell formation in both Leghorn hens and Tsaiya ducks. Therefore, a series of experiments was set up to determine the mechanism for magnesium and calcium in eggshell formation in these two laying fowls. This review will focus mainly on the experimental results obtained in my laboratory on the role of magnesium and calcium in eggshell formation in Tsaiya ducks and Leghorn hens.

DIETARY CALCIUM EFFECT ON CALCIUM AND MAGNESIUM CONTENT IN EGGSHELL

Calcium is the major component in an eggshell. There is also a small amount of magnesium in the eggshell. To see the possible effect of dietary calcium on the amount of magnesium in the eggshells of laying fowl, Chen and Shen (1989) designed an experiment to evaluate the effect of dietary calcium on eggshell calcium and magnesium deposition in both Leghorn hens and Tsaiya ducks. The data in Table 1 shows that the duck eggshells had significantly higher calcium content and significantly lower magnesium contents than chicken eggshells. The magnesium content in the duck eggshells was not affected by the levels of calcium in the diet. However, the magnesium content in Leghorn hen eggshells was significantly decreased ($p<0.05$), as the dietary calcium level was increased from 1 to 3%, and remained constant as the dietary calcium was increased

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Table 1. Influence of dietary calcium levels on the calcium and magnesium content in Tsaiya duck and White Leghorn hen eggshells

Dietary Ca	Calcium			Magnesium		
	Duck	Hen		Duck	Hen	
(%)	(%)			(%)		
1.0	38.6[a]	37.5[a]	*	0.12[a]	0.62[a]	*
2.0	38.1[ab]	36.5[ab]	*	0.14[a]	0.45[b]	*
3.0	37.1[c]	35.5[b]	*	0.13[a]	0.33[c]	*
4.0	37.5[bc]	36.4[ab]		0.12[a]	0.33[c]	*
5.0	38.6[a]	36.0[b]	*	0.13[a]	0.31[c]	*
Pooled SD	1.19	1.79		0.02	0.04	

[a-c.] Means on the same column without common superscripts differ significantly (p<0.05).

(Chen and Shen, 1989)

Table 2. Effects of dietary magnesium level on the eggshell breaking strength and the magnesium content in Tsaiya duck and Leghorn hen eggshells

Dietary Mg	3[rd] wk eggshell breaking strength		Eggshell magnesium	
	Duck	Hen	Duck	Hen
(mg/kg)	(kg/cm^2)		(%)	
690	4.70[a]	3.61[a]	0.115[a]	0.279[a]
1,070	4.77[a]	3.64[a]	0.114[a]	0.377[b]
1,690	5.07[a]	3.36[a]	0.113[a]	0.387[b]
2,150	4.66[a]	3.47[a]	0.116[a]	0.394[b]
2,380	4.84[a]	3.53[a]	0.123[a]	0.427[c]

[a-c] Means on the same column without common superscripts differ significantly (p<0.05).

(Ding and Shen, 1992.)

from 3 to 5%. This agrees with the report of Holder and Huntly (1978) who indicated that eggshell magnesium content was significantly higher in eggs from hens fed 2.5% calcium than those fed 3.5% calcium. The magnesium content in eggshells may have a relation to eggshell quality. From regression analysis for all egg samples, Chen and Shen (1989) found that there was a negative correlation between the eggshell magnesium content and eggshell thickness (r=-0.84, p<0.05 for hen's egg; r=-0.41, p<0.05 for duck's egg). Atteh and Leeson (1983) obtained similar results with hen's eggs. Conversely, Brooks and Hall (1955) observed that the stronger shells contained a higher magnesium concentration than weaker shells and suggested a direct connection between the magnesium content and the physical properties of hen's eggshells. Therefore, a question exists as to how the magnesium content in the eggshell influence shell quality.

THE EFFECT OF DIETARY MAGNESIUM LEVELS ON EGGSHELL QUALITY

The magnesium content in an eggshell may have effect on eggshell quality. Our previous study (Chen and Shen, 1989) showed that the magnesium content in eggshells had a negative influence on the quality of the eggshells in laying fowl. In this section, we will examine more data on the dietary magnesium effect on eggshell quality. Information on the influence of dietary magnesium content on Tsaiya duck eggshells has not been established.

The effects of dietary magnesium on the eggshell breaking strength and eggshell magnesium content are shown in Table 2. It is clear that the dietary magnesium within the levels used in the experiment did not result in any difference in the eggshell breaking strength in both ducks and hens. However, the duck eggshell had higher breaking strength than those of Leghorn hens. There were different effects from dietary magnesium levels on the magnesium content in Tsaiya duck and Leghorn hen eggshells. The eggshell magnesium content in the duck eggs was constantly maintained at 0.113-0.123% among the dietary magnesium levels studied. However, in Leghorn hens, an increase in the dietary magnesium levels resulted in a linear increase in the eggshell magnesium content from 0.279 to 0.427%.

The dietary magnesium levels significantly affected the plasma magnesium concentration in both Tsaiya ducks and Leghorn hens (Table 3). In Tsaiya ducks, the plasma magnesium content of the group fed magnesium 690 mg/kg diet was lower than that from the other treatments. This might mean that magnesium at 690 mg/kg diet was boardline as to the dietary requirement for magnesium although the egg production and egg weight were not affected. In Leghorn hens, the response was different, as the dietary magnesium levels were increased, there was an increase in the plasma magnesium content. This may indicate that the plasma magnesium concentration responds directly to the amount of magnesium absorbed from the intestine in hens.

According to Stafford and Edwards (1973), a magnesium deficiency in the diet would result in eggshell quality impairment and a level of magnesium at 486 mg/kg

Table 3. Effect of dietary magnesium content on the concentration of plasma magnesium in Tsaiya ducks and Leghorn hens

Dietary Mg	Plasma magnesium	
	Duck	Hen
(mg/kg)	(mg/dL)	
690	3.73[a]	1.66[a]
1,070	4.66[b]	3.19[b]
1,690	4.97[b]	3.55[c]
2,150	4.91[b]	3.82[cd]
2,380	4.90[b]	4.03[d]

[a-d] Means on the same column without common superscripts differ significantly (p<0.05).

(Ding and Shen, 1992.)

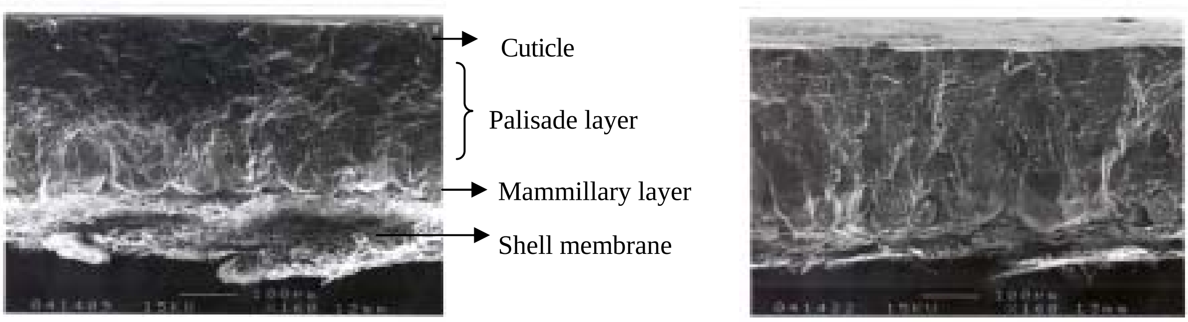
diet was believed to meet the minimal requirement for Leghorn hens. No more benefit could be obtained by further magnesium addition. Our results (Ding and Shen, 1992) on eggshell strength in both ducks and hens showed a similar outcome. The normal plasma magnesium concentration in laying hens was located between 2.4 and 4.8 mg/dL as indicated by Stafford and Edwards (1973). According to this criterion, hens fed a basal diet containing magnesium 690 mg/kg diet (Ding and Shen, 1992) exhibited hypomagnesemia (1.66 mg/dL). This problem could be overcome by higher levels of dietary magnesium. To maintain optimal plasma magnesium content, the dietary magnesium level for laying hens should be higher than 500 mg/kg diet as recommended by NRC (1994).

STRUCTURE OF EGGSHELL IN BOTH DUCK AND HEN

The ultrastructure and crystal structure of Tsaiya duck and Leghorn hen eggshells were compared in our laboratory (Chen and Shen, 2000). The eggshell crystal structure was detected using an X-ray diffractometer. The eggshell ultrastructure was observed using scanning electron microscopy. Results showed that the eggshell crystal structures were nearly pure calcite ($CaCO_3$) in both the duck and hen eggs. Our results confirm the studies by Solomon (1991) and Dennis et al. (1996) indicating calcite as the crystal structure of the Leghorn hen eggshell. The eggshell ultrastructure is shown in Figure 1, obtained from normal eggs (Chen and Shen, 2000). Under low magnification (160×), there was not much difference in scanning electron micrograph between duck and hen eggshell. However, as the palisade region of eggshell was magnified to 5000× (Figure 2), the palisade layer was found to be in a compact form for Tsaiya ducks. There were hallow vesicles throughout the palisade layer for the chicken eggshell. This phenomenon clearly indicates that Tsaiya duck eggshells possess a more compact palisade layer, which may be the reason for the stronger duck eggshell strength.

MAGNESIUM AND CALCIUM DEPOSITION MODEL IN EGGSHELL

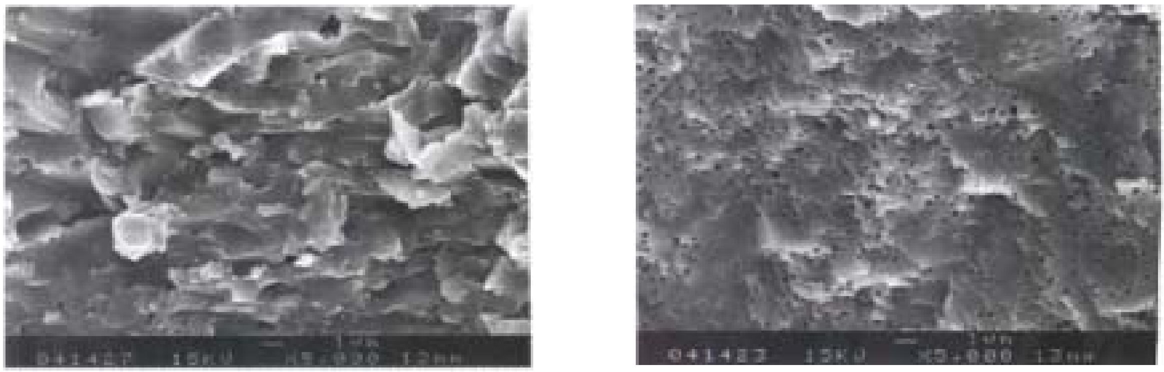
Our previous studies concluded that the magnesium content in eggshell was lower in duck eggs than in chicken eggs. This precipitated our interest in investigating an eggshell magnesium and calcium deposition model. The electron probe microanalyzer (EPMA) was used to scan the magnesium and calcium distribution across the eggshell cross section. The results are shown in Figure 3 and 4. Figure 3 shows that the magnesium deposition model in eggshell is different in ducks and hens. In the chicken eggshell, there are two peaks in the magnesium distribution. The first peak is in the early stage of eggshell formation with a higher amount of magnesium deposition in the mammillary layer. The second peak arises in the latter stage of eggshell formation in which more magnesium is deposited in the outer portion of the palisade layer. However, in duck eggshells, there is only one peak appearing on the inner side of the eggshell. The amount of magnesium deposition declines thereafter until the end of eggshell formation. In the calcium deposition model (Figure 4), calcium is deposited constantly throughout the eggshell formation period. The models are similar for ducks and hens. The outcomes were similar to the results obtained by Waddell et al. (1989, 1991) who fed normal or low magnesium diets (magnesium 207 or 132 ppm) to ISA Brown hens and obtained constant calcium deposition in the eggshell throughout the egg formation stage.



(A) Brown Tsaiya duck
ST=4.91 kg TK=394 μm

(B) White Leghorn hen
ST=4.07 kg TK=381 μm

Figure 1. Scanning electron micrographs (SEM; 160×) of the cross section in normal eggs from Brown Tsaiya duck (A) and White Leghorn hen (B). (ST = shell strength; TK = shell thickness without shell membrane). (Chen and Shen, 2000)



(A) Tsaiya duck (B) Leghorn hen

Figure 2. Scanning electron micrographs (SEM; 5000×) of palisade region in eggshell from (A) Brown Tsaiya duck and (B) White Leghorn hen. (Cited from Chen and Shen, 2000)

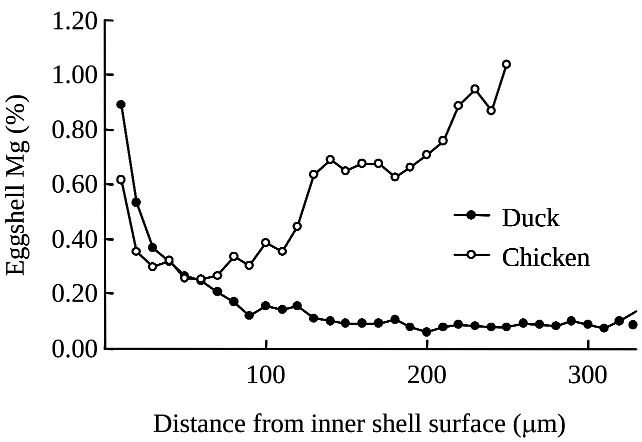


Figure 3. Magnesium deposition models for eggshells of Tsaiya duck (●) and White Leghorn hen (○) fed a normal diet. (Chen, 2000)

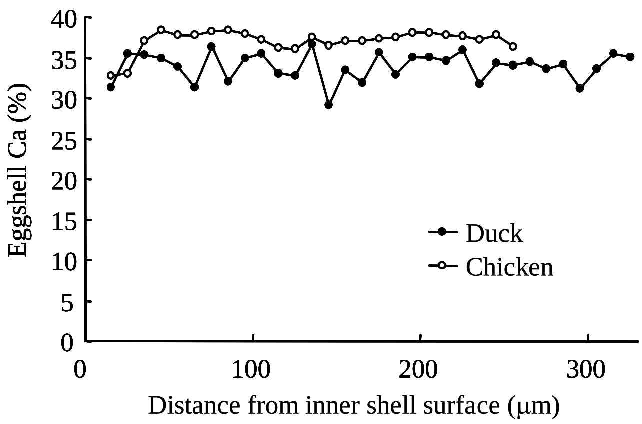


Figure 4. Calcium deposition models in eggshells of Tsaiya duck (●) and White Leghorn hen (○) fed a normal diet. (Chen, 2000)

DIETARY CALCIUM EFFECT ON CARBONIC ANHYDRASE ACTIVITY IN SHELL GLAND MUCOSA

The eggshell is formed in shell glands of laying birds. The carbonic anhydrase activity in the shell gland mucosa may be different in Tsaiya ducks and Leghorn hens. Wei and Shen (1991) studied the effect of dietary calcium levels (1 to 5%) on the carbonic anhydrase activity in the shell gland mucosa of laying Tsaiya ducks and Leghorn hens. The results showed that as the calcium intake was sufficient for both ducks and hens to maintain normal egg production, the dietary calcium content had no effect on the carbonic anhydrase activity in the shell gland mucosa. Some ducks and hens fed diets containing 1% calcium stopped laying with shell gland atrophy because of low calcium intake. The enzyme activity in hens with shell gland atrophy was significantly lower than that for normal hens, but was undetectable in ducks. Both specific and total carbonic anhydrase activities in the shell gland mucosa in ducks were higher than that in hens. It was concluded that the higher carbonic anhydrase activity might be the reason for ducks to produce better shell quality than hens.

DIETARY MAGNESIUM AND CALCIUM ON MAGNESIUM DEPOSITION MODEL IN EGGSHELL

The dietary magnesium and calcium contents may have an effect on the magnesium deposition model in Tsaiya duck and Leghorn hen eggshells. Hence, an experiment was conducted in our laboratory (Chen, 2000) to investigate the influence of dietary magnesium and calcium levels on the eggshell magnesium deposition model and its effect on eggshell quality. In the experiment, birds were fed four experimental diets including: (1) normal (Ca 3.42%, Mg 1984 ppm); (2) low-Ca (Ca 1.17%, Mg 1,917 ppm); (3) low-Mg (Ca 3.40%, Mg 138 ppm) and (4) low-Ca, high-Mg (Ca 1.23%, Mg 5,943 ppm) for 15 days. The results showed that eggshell strength and eggshell thickness were all higher in ducks than in hens given the different experimental diets. Although the eggshell strength and thickness were drastically decreased 40-50% after feeding three days on low-Ca, low-Mg or low-Ca, high-Mg diets, the egg production rate was still maintained at around 63-70% in hens. However, the ducks stop laying after they were fed low-Ca diet for 6 days. It appeared that the mechanism in which the low-Ca diet caused egg production inhibition might be different in ducks and hens. For ducks, when the plasma calcium content was low, the ducks would stop laying in order to protect themselves.

In the experiment, the relationship between plasma magnesium and eggshell magnesium content was also determined. In the normal group, the plasma magnesium content in the hens was lower than that in ducks, but the eggshell magnesium content in hens was about four fold higher than that for ducks. As the hens were given low-Ca diet, the plasma magnesium content increased and the eggshell magnesium content also increased to 0.554%. However, in ducks, the plasma magnesium content declined and the eggshell magnesium was still maintained at low levels of 0.12-0.13%. As the birds were fed a low-Mg diet, the plasma magnesium content would decline to about 60% on the 3rd day and remain low thereafter. However, the eggshell magnesium content decreased with time as the hens were fed the low-Mg diet, but not in ducks, as they remained constant until the end of the experiment. This once again means that the magnesium deposition mechanism is different between hens and ducks. As the birds were fed low-Ca, high-Mg diet, the plasma magnesium concentration increased as well as the eggshell magnesium content in Leghorn hens. However, in ducks, the eggshell magnesium content still remained at low levels. As for the relationship between plasma calcium concentration and eggshell calcium content, plasma calcium concentration for ducks was 6-10 mg/dL higher than those of hens for 4 types of diets used in the experiment. The eggshell calcium content for ducks was about 35-36%, which was also higher than hens with 32-33% indicating better calcium deposition in the eggshell in ducks.

Magnesium and calcium deposition models were also measured using electron probe microanalyzer in this experiment. The results indicated that the calcium deposition model did not change in both birds fed diets containing different levels of calcium and magnesium. This outcome is similar to the results by Waddell et al. (1989, 1991) who showed that the eggshell calcium distribution in ISA Brown layer was fairly constant in bird fed diets with different levels of magnesium. However, the magnesium deposition model was very different for ducks and hens (Figure 5). Figure 5 shows that the magnesium deposition

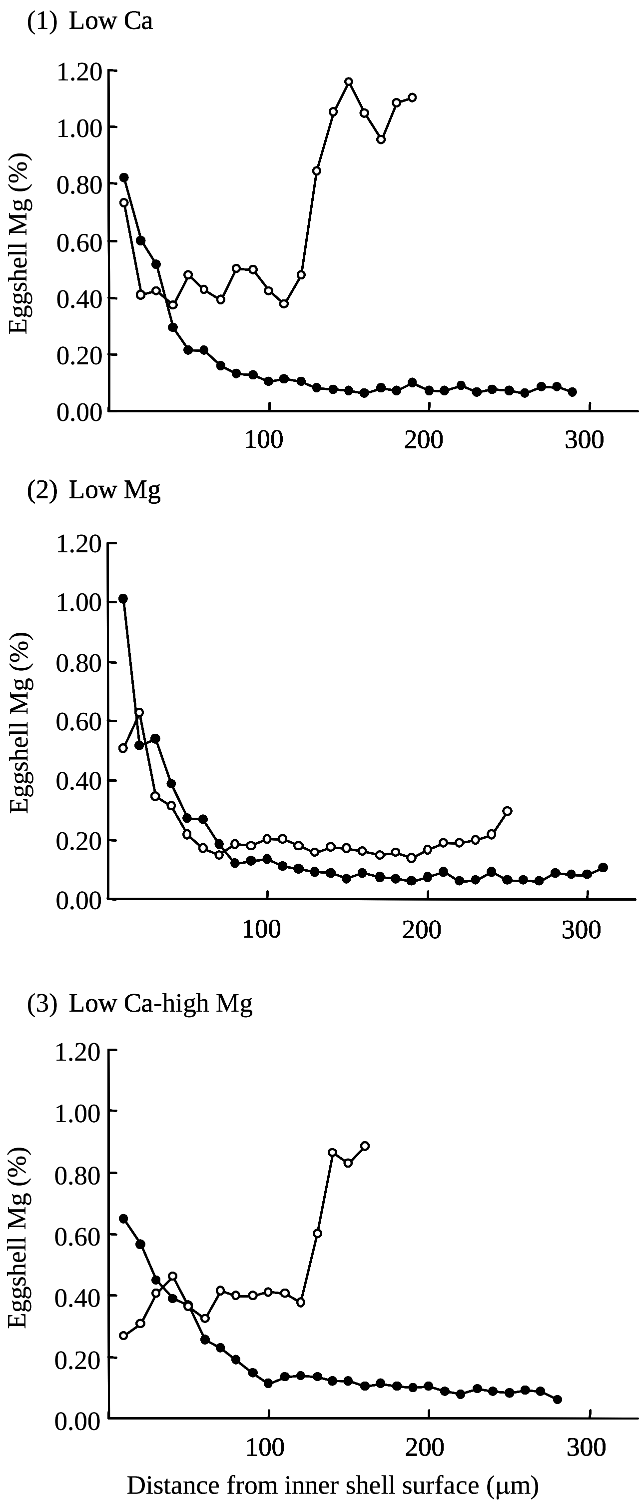


Figure 5. Magnesium deposition models for eggshells of Tsaiya duck (●) and white Leghorn hen (○) fed diets containing different levels of magnesium and calcium. (Chen, 2000)

model appears two peaks in the chicken eggshell, one in the inner layer and the other at the outer surface of eggshell, except with low-Mg treatment only one peak occurred in the inner layer. However, there was only one peak in the inner layer for the duck eggshell. The results indicate that the mechanism for magnesium deposition in eggshell is different between ducks and hens. This may be one of the reasons that duck eggs have a stronger eggshell than hen eggs. From our analysis of the available data on the correlation of magnesium content in eggshell and eggshell thickness, it could be concluded that there was a negative correlation between eggshell magnesium content and eggshell thickness (r=-0.485 and $p<0.05$ for hen; r=-0.523 and $p<0.05$ for duck). Therefore, the amount of magnesium deposited in the eggshell might have an effect on eggshell quality.

CALCIUM AND MAGNESIUM TRANSPORT THROUGH SHELL GLAND AT VARIOUS STAGES OF EGGSHELL FORMATION

The eggshell is formed in the shell glands of laying birds. The calcium and magnesium transport through the shell gland mucosa may have a great influence on eggshell quality. Chen and Shen (2001) determined the calcium and magnesium content in the shell gland mucosa at various stages of eggshell formation and found that the shell gland mucosa in Tsaiya ducks contained lower calcium and higher magnesium than the shell mucosa in Leghorn hens, but the uterine fluid in Tsaiya ducks contained higher calcium and lower magnesium than that in Leghorn hens. It is interesting to find that the changes in magnesium concentration in the uterine fluid during eggshell formation (Figure 6) corresponded closely with the magnesium deposition models for ducks and hens. This phenomenon clearly indicates a different time course in magnesium deposition in the eggshells of these two laying birds.

The ATPase activity in the shell gland mucosa is closely related to the calcium transport during eggshell formation in laying birds. Therefore, ATPase activity was measured to determine if there was a difference between Tsaiya ducks and Leghorn hens. Figure 7 shows that shell gland mucosa ATPase specific activities exhibit only minor changes ($p>0.05$) during eggshell formation in both birds. Mg^{2+} added to the incubation medium improved the uterus mucosa ATPase activity in Leghorn hens, but not in Tsaiya ducks. The activated efficiency of ATPase in hens was Ca^{2+}-Mg^{2+}>Mg^{2+}>Ca^{2+}.

In conclusion, Tsaiya ducks could retain higher magnesium and lower calcium content in the shell gland mucosa and secret less magnesium and more calcium into the shell gland lumen for eggshell deposition. The specific activity of ATPase remained fairly constant during the

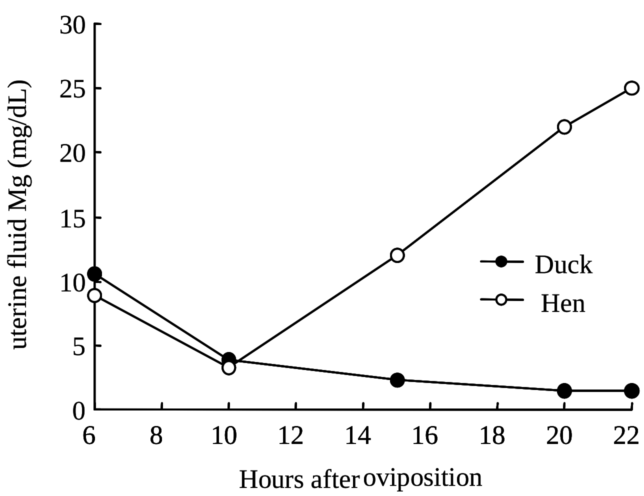


Figure 6. Uterine fluid magnesium concentration during eggshell formation in Tsaiya duck and Leghorn hen. (Chen, 2000)

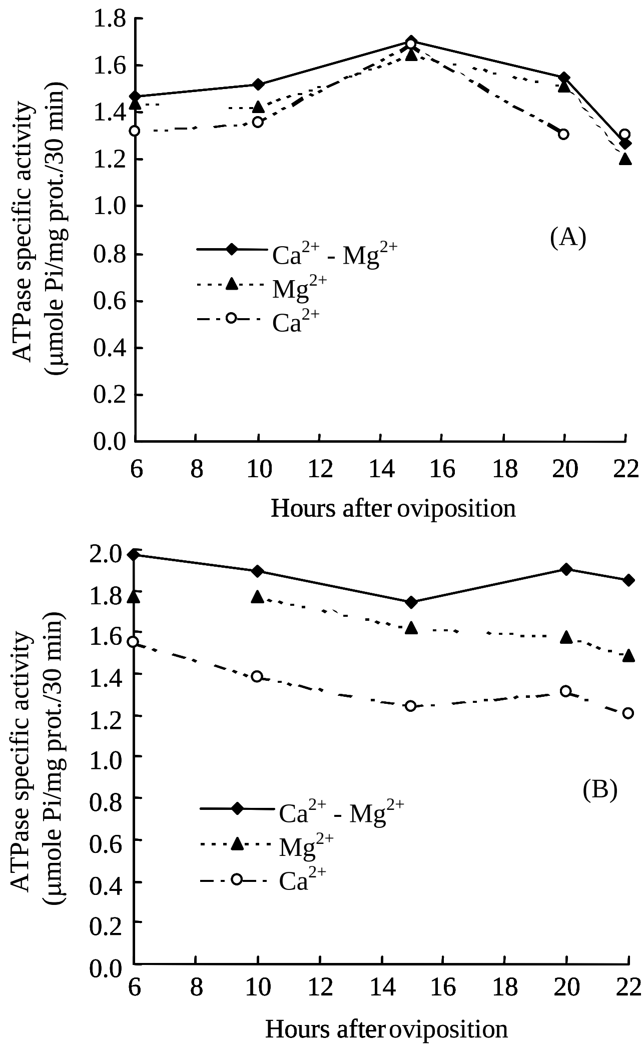


Figure 7. Variations of Ca-Mg-, Mg- and Ca-ATPase activities during eggshell formation in Tsaiya ducks (A) and Leghorn hens (B). (Chen, 2000)

eggshell formation stage indicating continuous calcium transport into the shell gland lumen for eggshell formation. The magnesium transport mechanism in the shell gland mucosa is worth further study.

CONCLUSION

The role of calcium and magnesium in eggshell formation in laying birds was studied. The duck eggshells contained higher calcium and lower magnesium than hen eggshells. In ducks, the magnesium content in the eggshell was not affected by dietary magnesium levels (690-2380 ppm). However, in hens, eggshell magnesium content increased with the increasing levels of dietary magnesium. The palisade layer (5000×) of the eggshell was compact for ducks, but had many hollow vesicles in the chicken eggshell. The magnesium deposition model exhibited one peak for ducks and two peaks for hens. The specific and total carbonic anhydrase activities in the shell gland mucosa of ducks were higher than those for hens. Ducks could retain higher magnesium and lower calcium in the shell gland mucosa and secret less magnesium and more calcium into the shell gland lumen for eggshell deposition. The magnesium content in duck eggshells was much lower than that for hens indicating that the eggshell magnesium content might have an effect on eggshell quality.

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