The effect of sodium chloride on eggshell quality in laying hens - A review


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Abstract

Sodium chloride, NaCl, has been shown to have a severe adverse effect on shell quality in birds when received in drinking water. This is a major problem due to an increase of breakage of eggs and great economic losses in commercial egg production. This review focuses on what has been written about the effect of NaCl on eggshell quality in the laying hen, with emphasis on the function of the enzyme carbonic anhydrase (CA) in shell formation. CA is important for the shell formation through the catalyzation of the reaction $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$ in the shell gland. HCO_3^- concentration in the shell gland has been shown to be coupled to the secretion of Ca^{2+} from the blood to the shell gland lumen and together they form $CaCO_3$. Studies have shown that NaCl inhibits the activity of CA which causes a reduced concentration of HCO_3^- and Ca^{2+} in the shell gland, resulting in a reduction of the shell quality. The NaCl affects several shell quality measurements such as shell weight, shell breaking strength and shell thickness. The effect depends on the hens' age and strain, and there are also individual differences. Older hens are more sensitive to saline drinking water than pullets and the strain Isa Brown is less sensitive than the cross between White Leghorn and New Hampshire. The exact effect of NaCl on the eggshell formation is yet not fully understood and further research is needed. The best remedy for hens receiving saline water is desalination of the water, but preventative treatments such as supplementing the diet or water with ascorbic acid or zinc has also proven beneficial for the shell quality.

Sammanfattning

Natrium klorid i dricksvattnet har visat sig ge negativa effekter på skalkvalitet hos höns. Detta är ett allvarligt problem då detta ger en ökning av förstörda ägg vilket kan resultera i stora ekonomiska förluster inom äggproduktionen. Denna litteraturstudie sammanställer vad som tidigare skrivits om effekten av NaCl på skalkvalitet hos den producerande hönan, med betoning på funktionen av enzymet karbanhydras (KA). KA är viktig för skalbildningen genom sin katalys av reaktionen $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$ i skalkörteln. Utsöndringen av Ca^{2+} från blodet till skalkörteln är kopplad till koncentrationen av HCO_3^-, tillsammans bildar de $CaCO_3$. Studier har visat att NaCl inhiberar aktiviteten av KA i skalkörteln, vilket resulterar i en minskad koncentration av HCO_3^- och Ca^{2+} som kan leda till en reducerad skalkvalitet. NaCl påverkar flera kvalitetsmått så som skalvikt, skalstyrka och skaltjocklek. Effekten har visat sig vara beroende av hönans ålder och ras. Det finns även individuella skillnader i respons. Äldre hönor är känsligare för vatten med hög saltkoncentration än vad unghöns är och rasen Isa Brow är mindre känslig än korsningen mellan White Leghorn och New Hampshire. Den exakta effekten av NaCl på skalbildning är ännu inte helt klargjord och vidare forskning behövs. Det bästa sättet att undvika negativa effekter av NaCl på skalkvaliteten för höns som dricker saltvatten är avsaltning av vattnet. Förebyggande behandlingar såsom tillsats av askorbinsyra eller zink till fodret eller vattnet har också visat sig vara värdefulla.

Introduction

The quality of the hens eggshell is important. Shells that are too weak risk breaking during incubation or handling. This could have a significant economic impact for egg producers. Studies have shown that sodium chloride (NaCl) has a major effect on the quality of the eggshell, such as shell breaking strength, shell thickness and shell weight. Relatively high concentrations of NaCl in the drinking water can even result in hens being unable to deposit a shell on the egg during its passage through the shell gland in the oviduct (Balnave et al. 1989).

But NaCl is also essential for poultry. A diet with no added salt will result in a rapid decline in egg production (Begin & Johnson, 1976). Depending on in what form the NaCl is consumed it affects the hen in different ways. Yoselewitz & Balnave (1989b) showed that the incidence of cracked, broken or soft shelled eggs is significantly greater when the NaCl is given in the drinking water compared to in the diet.

Many countries use underground water as the main water supply for poultry. This water is usually high in dissolved mineral salts. Concentrations as high as 570mg/l of sodium and 2000 mg/l of chloride have been recovered in bore water (as reviewed by Balnave, 1993). Several studies have been made on the effect of NaCl in drinking water, and the results shows a reduction of calcium-binding protein (CaBP) (Balnave & Zhang, 1993) and the enzyme carbonic anhydrase (CA), which is important for shell formation (Yoselewitz & Balnave, 1989a; Balnave, 1993). This might have a negative impact on the egg shell quality.

The purpose of this literature review is to compile what has earlier been written about the effect of NaCl on eggshell quality in the laying hen, with emphasis on the function of carbonic anhydrase in shell formation.

The Egg

It takes 24-26 hours for the egg to travel through the oviduct and fully develop. The egg consists of yolk, albumen, organic matrix and a crystalline shell (figure 1). The yolk provides the embryo with lipids and many of the proteins that are needed during the embryonic growth, while the albumen prevents microorganisms to invade the yolk. The albumen also supplies the embryo with water, protein and minerals during the development. The organic matrix is composed of two shell membranes with mammillary cores, shell matrix and the cuticle. The inner and outer layers of the shell membranes are semi permeable, and allow passage of gases and water. The mammillary cores are the initial site of calcification and consist of protein and carbohydrates. The crystalline shell (consisting of 97 % $CaCo_3$) is divided in two layers; the mammillary knob layer and the palisade layer. Finally the shell is covered by the cuticle (figure 1) (Johnson, 2000).

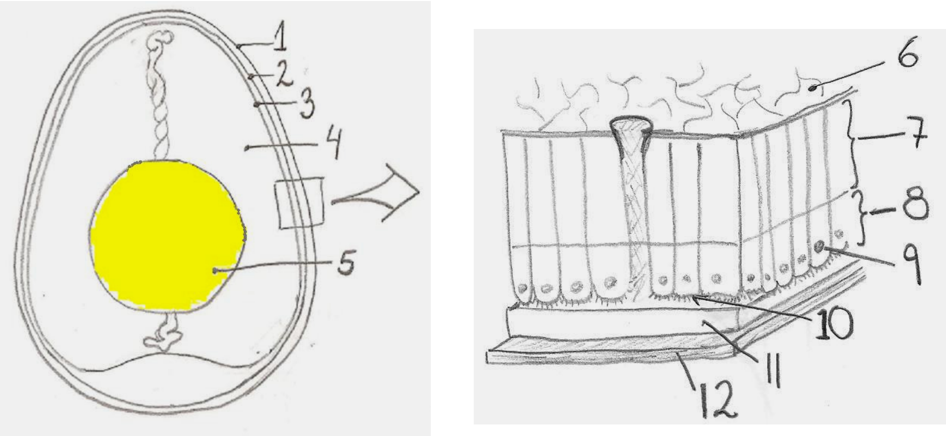

Figure 1: A schematic picture of the egg and the eggshell. 1) Eggshell 2) Outer membrane 3) Inner membrane 4) Albumen 5) Yolk 6) Cuticle 7) Palisade layer 8) Mammillary layer 9) Mammillary cores 10) Calcium deposits 11) Outer membrane 12) Inner membrane. Based on Johnson (2000).

Shell formation

The shell is produced in the part of the oviduct that is known as the shell gland. First, a process known as plumping (dilution of the albumen) occurs by the passage of water and ions to the albumen which doubles its size. Plumping stretches the shell membranes and the distance between the tips of the mammillary cores increases. A mammillary knob layer is formed by outward crystallization of the mammillary cores (see figure 1), this occurs in the shell gland during the first five hours of calcification. The calcified crystals together with the matrix form the palisade layer of the shell, which consists mainly of crystalline calcium carbonate ($CaCO_3$) in the form of calcite. This layer is arranged in columns placed directly over the mammillary knobs. The calcium carbonate deposition takes around 15 hours. The final layer on the egg is the cuticle, which is a thin waxy coat on the surface of the egg that is formed during the last 30 minutes prior to oviposition. It consists of protein, polysaccharides and lipids and protects the egg from water evaporation and microbial invasions (Johnson, 2000). After 20 hours in the shell gland the egg moves towards the vagina driven by oviductal tissue contractions and the egg is laid.

Carbonic anhydrase and shell formation

CA is an enzyme family with 16 different isozymes presently known (Singer et al., 2009), though only 13 have catalytic activity (Hilvo et al., 2009). The isozymes can be organized according to their cellular localization. For example CA I-III are found in the cytosol, CA IV, IX, XII, XIV and XV are membrane-bound and CA V mitochondrial (Hilvo et al., 2009). Gutowska & Mitchell showed as early as 1945 how important CA is for the formation of the eggshell. They measured the activity of CA in the shell gland of hens laying strong and smooth shelled eggs and hens laying soft-shelled eggs. A significant decrease in CA activity in the shell gland in hens laying soft-shelled eggs was observed. They also found that inhibiting CA resulted in a decrease in shell quality. This shows that the quality of the eggshell may be directly dependent upon the activity of CA in the shell gland. All hens were being fed a calcium rich diet, which indicate that the reason for hens laying soft-shelled eggs was not calcium deficiency. It was suggested that it was rather a lowered ability to bind calcium due to a lower concentration of carbonate ions. A decrease in CA activity has also been suggested to cause a reduced CO_2 diffusion, which may result in a reduction of HCO_3^- transfer to the shell gland lumen (Berg et al., 2004). This might reduce the availability of calcium in the shell gland, since it is coupled to the lumenal HCO_3^- concentration (Eastin & Spaziani, 1978; Lundholm, 1990), resulting in a reduced shell quality.

The eggshell consists mainly of $CaCO_3$ which is formed by Ca^{2+} and HCO_3^-. At puberty, the liver secretes CaBP, which increases the ability of the blood to transport Ca^{2+}. The Ca^{2+} comes from bone resorption and absorption from the intestines and it is transported by the blood across the epithelium to the shell gland lumen (Sjaastad et al., 2007). This transport is partly driven by the activity of CA (as reviewed by Bar, 2009). CA is a key enzyme in the eggshell formation of the domestic hen. It can be found in the glandular cell membranes as well as in capillaries, the most activity in the shell gland has been shown to reside in the capillary endothelium (Berg et al., 2004). CA is a zinc-depending enzyme that catalyzes the reaction $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. By increasing the carbonate availability a fast calcite crystal growth occurs (Fernandez et al., 2004). The formation of HCO_3^- is essential for the deposition of calcium carbonate in the shell, where it acts as the sole counter ion for Ca^{2+}. By binding to the carbonate ion Ca^{2+} is removed from the lumen fluid. This may result in a luminal Ca^{2+} gradient, which gives a passive diffusion of Ca^{2+} from blood to the shell gland lumen (as reviewed by Bar, 2009).

Effect of NaCl in the laying hen

NaCl is an essential mineral for hens. A low-salt diet causes a significant depression in egg weight and a reduction in feed consumption. The mortality of hens on a low-salt diet (containing 0.125 percent added salt) is significantly greater than that of hens on a salt adequate diet (containing 0.25 percent salt) (Begin & Johnson, 1976). The hatchability of the eggs in birds receiving a salt sufficient diet is significantly better than in birds on a low salt diet (Nestler, 1943). But NaCl received through the drinking water can have severe adverse effects on the laying hen. The activity of CA in the shell gland in birds that has received saline drinking water has been shown to be significantly lower compared to the CA activity in hens drinking town water (Yoselewitz & Balnave, 1989a; Balnave & Zhang, 1993). This effect can be explained by the fact that an excess of chloride ions inhibits the CA activity (Dionisiosese & Miyachi, 1992). This results in a limited supply of bicarbonate ions to the lumen of the shell gland, which also limits the uptake of calcium to the shell gland (Chen & Balnave, 2001).

The effect of the NaCl has been shown to depend on age and strain and if it is obtained from drinking water or diet. When similar intakes of NaCl is consumed through drinking water and through diet a significant decrease in eggshell quality can be seen in hens receiving NaCl through drinking water but not through the diet (Yoselewitz & Balnave, 1989b; Pourreza et al., 1994). There are also individual differences in the response. Some hens continue to lay eggs with good shell quality even when receiving saline drinking water, but increasing number of hens are affected as the NaCl concentration in the drinking water increases (Balnave et al., 1989; Yoselewitz & Balnave, 1989b; Pourreza et al., 1994).

Age

Sensitivity towards saline water has been shown to increase with age (Yoselewitz & Balnave, 1989c; Hadziosmanovic et al., 1997; Balnave & Zhang, 1998) and there is an increase in the percentage of damaged eggs toward the end of the laying period (Hadziosmanovic et al., 1997). Pullets that are given saline drinking water during growth and before sexual maturity do not show any negative effect on shell quality as shown in table 1, cited from Yoselewitz & Balnave, 1989c (Yoselewitz & Balnave, 1989c; Balnave & Zhang, 1998). Young hens drinking saline water from the start of lay does sometime not show a significant increase in shell defects until a later age (29 weeks) (Yoselewitz & Balnave, 1989c). If the saline water is replaced with town water before 29 weeks of age the negative effect might not show until 55 weeks of age (Balnave & Zhang, 1998). This suggests that these hens has not fully recovered from the NaCl treatment, and that hens that are given saline drinking water for only a few weeks in early- or mid-lay suffer consequences at a much later age.

Birds drinking saline water during the peak of lay (29-33 weeks of age) show a significant reduction of shell quality, but when the saline water is replaced with town water the shell quality returns to normal. It appears that it is possible to reduce the incidence of shell defects that is caused by saline drinking water down to control levels in hens in early lay by giving them town water again (Yoselewitz & Balnave, 1989c). Older hens (40 weeks and onwards) drinking saline water also shows a decrease in shell quality (Balnave & Zhang, 1998), but the recovering response when drinking town water again has not been observed in this age group (Balnave & Yoselewitz, 1987; Yoselewitz & Balnave, 1989b,c). Young hens (up to the peak of lay) have a greater ability to recover from the adverse effect of saline drinking water then older birds (Yoselewitz & Balnave, 1989c).

Table 1: Significant increase in shell defects in pullets between 15 and 45 weeks of age and receiving town and saline water (cited from Yoselewitz & Balnave, 1989c)

Town water (weeks)	**Saline water (weeks)**	**Significant increase in shell defects**
15-33	—	no
18-33	15-18	no
1st egg-33	15-1st egg	no
15-1st egg	1st egg-33	yes
—	15-33	yes
40-45	—	no
—	40-45	yes[1]

[1] Value significant compared to hens receiving town water only

Strain

The sensitivity to saline water has been shown to vary a lot between different strains (Balnave, 1993; Hadziosmanovic et al., 1997). One example of this is the strain Isa Brown in which no significant increase in eggshell defects has been observed (Hadziosmanovic et al., 1997; Chen & Balnave, 2001). The cross between White Leghorn and New Hampshire or Australorps seems to be more sensitive to saline drinking water compared to Isa Brown and White Leghorn (Balnave et al, 1989; Yoselewitz & Balnave, 1989a,b,c; Balnave et al., 1991; Moreng et al, 1992; Balnave & Zhang, 1992, 1993, 1998). There also seems to be individual differences regarding the sensitivity to saline water within the strains. The strain White Leghorn has shown both significant reduction in shell quality (Pourreza et al., 1994) as well as not being influenced by waterborne NaCl (Damron, 1998). Concentrations as low as 200 mg NaCl/l drinking water increase the number of damaged eggshells significantly in some studies (Balnave & Yoselewitz, 1987) while others report of using concentrations up to 2 g NaCl/l with no significant effect in regards to shell quality (Balnave & Zhang, 1993; Damron, 1998; Chen & Balnave, 2001).

Effects of saline water on the availability of Ca^{2+} and HCO_3^- in the shell gland

Increased concentrations of NaCl in the drinking water of laying hens have been shown to lead to an increase of both calcium and phosphorus in the plasma (Pourreza et al., 1994). Despite this raise of calcium, shell calcium and shell thickness is reduced. This indicates that the uptake of calcium by the shell gland might be impaired. In hens receiving saline water the concentration of CaBP in the shell gland is significantly decreased as well as the concentration of HCO_3^-, Ca^{2+} and partial pressure of CO_2 in the shell gland fluid (Balnave & Zhang, 1993; Balnave et al., 1989). This effect appears to be long lasting since replacing the saline water with town water has little effect on the reduction of the concentration of HCO_3^-, Ca^{2+} and partial pressure of CO_2 even after several weeks.

A reduced supply of HCO_3^- in the shell gland is a limiting factor for shell quality since it is needed for the synthetisation of calcium carbonate. The degree of shell damage is negatively correlated with the concentration of HCO_3^- in the shell gland fluid (Balnave et al., 1989). Furthermore, oviposition value (residence time of the egg in the oviduct) is positively correlated with the HCO_3^- concentration in the shell gland fluid. A decrease in HCO_3^- concentration would therefore result in a reduction in time that the egg remains in the shell gland. The shorter the time, the smaller the amount of calcium carbonate that can be deposited as shell (Balnave et al., 1989). In a study made by Balnave et al. (1989) the production of soft shelled eggs went up to 17.3% in hens receiving 2g NaCl/l drinking water, compared with less than 1% in hens receiving town water. This indicates that the mechanism of shell formation in hens receiving saline water may be affected so severely that some individuals are unable to deposit a shell on the egg during its passage through the oviduct.

Effect of NaCl on eggshell quality

Shell quality has been shown to decrease as the concentration of NaCl in the drinking water increases (Pourreza et al., 1994). A significant linear relation has been shown between shell quality measurements and the concentration of NaCl in the drinking water. A concentration of 600 mg NaCl/l increases the incidence of damaged shells three-folded in domestic fowl. It also reduces shell breaking strength, shell thickness, shell weight, shell weight/egg weight and shell weight/unit surface (Balnave & Yoselewitz, 1987). Balnave & Zhang (1993) found only an increased incidence of reduction in shell breaking strength in hens receiving NaCl through the water. The authors speculate that this decrease, in the absence of reduction in the amount shell deposited, points towards that NaCl influences the structural organization of the shell.

Drinking water containing NaCl results in a long-term significant decrease of shell quality, and it is especially noticeable towards the end of lay (Balnave & Zhang, 1998). Normally eggshell quality improves after a rest of lay, but this does not occur if the bird has been drinking saline water (Balnave & Yoselewitz, 1987). The decrease in shell quality appears not to be related to a decrease in calcium intake or increased egg production (Balnave & Yoselewitz, 1987; Moreng et al., 1992; Pourreza et al., 1994; Kalafalla & Bessei, 1997).

Preventions and remedial treatments

Shell thickness can not be improved by reducing or eliminating dietary NaCl for hens receiving saline drinking water (Yoselewitz & Balnave, 1989b; Pourreza et al., 1994). But different kinds of preventions and remedial treatments do exist. For example, dietary bicarbonate supplement has been shown to improve the shell quality in hens under heat-stress, as long as the hen has access too feed during the period of eggshell formation (Balnave & Muheereza, 1997). Poultry under heat stress suffers from hyperventilation, resulting in a shortage of CO_2. This reduces the amount HCO_3^- that can be formed, resulting in poorer shell quality. As for hens drinking saline water a desalination of the drinking water may be the best remedy (Balnave, 1993), but supplementing ascorbic acid (AA) or zinc to the diet has also been proven to have beneficial effects on eggshell quality.

Ascorbic acid

Although the mechanism of AA remains to be clarified, it has been shown that the adverse effect of saline drinking water can be reduced by adding the vitamin AA to the diet or drinking water. The effect of AA depends on the concentration used. There is a significant correlation between the concentration of AA supplemented to the diet of hens drinking saline water and shell quality (Balnave & Zhang, 1992). AA has a positive effect on the metabolic rate of laying hens. Hens exposed to environmental stress (e.g. high temperature) is afflicted with reduced shell thickness, but supplementing their diet with AA significantly increases the shell thickness again. It is speculated that this increase is due to an increase in feed intake and improved efficiency of calcium utilization. Even hens living under no environmental stress benefits significantly from dietary AA in regard to shell thickness (Thornton & Moreng, 1959). It appears that AA is more efficient as a preventative treatment than a remedial treatment. Birds drinking water supplemented with NaCl and AA simultaneously shows a significant reduction in shell defects and a significant increase in eggshell quality. Balnave et al. (1991) did, however, not find any significant reduction in shell defects or increase in shell quality when AA was supplemented to hens that had previously received saline water and were laying eggs with defective shells.

In contrast to the findings above Kalafalla & Bessei (1997) could not observe any significant improvement of eggshell quality measures in hens drinking saline water and receiving AA. The overall mean of shell thickness was higher in hens drinking water with AA though. A reduction of defective shells was observed, but the result was not statistically significant.

Zinc

Carbonic anhydrase is a zinc dependent enzyme (as reviewed by Chen & Balnave, 2001). It has been shown that supplementing the diet with zinc-EDTA increases the activity of the enzyme significantly and reduces eggshell defects, such as soft, broken, cracked or deformed shells, in hens drinking saline water (Balnave & Zhang, 1993). The shell breaking strength is also significantly improved. Supplementing the diet with Zinc sulphate or Zinc methionine has also been shown to increase the concentration of CaBP and reduce the shell defects noticeably (Balnave & Zhang, 1993). Shell weight and shell weight/unit of surface area can also be improved by adding zinc methionine to the diet (Moreng et al., 1992).

Discussion

The understanding of the effect of NaCl in the laying hen is of great importance. NaCl is essential for poultry, but the adverse effect on shell quality may lead to an increase of egg breakage during incubation or handling. This can cause great economic losses in commercial egg production. The purpose of this literature review was to summarize what has been written about the effect of NaCl on eggshell quality in the laying hen, with emphasis on the function of carbonic anhydrase in shell formation.

Hens drinking saline water can suffer long-term negative effects in shell quality (Balnave & Zhang, 1998), even if the water is replaced with town water after a short period (Balnave & Yoselewitz, 1987; Yoselewitz & Balnave, 1989b,c; Balnave & Zhang, 1998). The difference in how NaCl affects the hen is depending on age, strain and individual responses within the hen. Bearing in mind that pullets are able to recover from the negative effects of saline water if they receive it before sexual maturity (Yoselewitz & Balnave, 1989c; Balnave & Zhang, 1998) it might be possible to “save” these young birds by switching to town water, or supply them with a suitable preventative treatment. Some strains of poultry are more sensitive towards drinking water supplemented with NaCl than others. By using these less sensitive hens in the areas which have high concentrations of NaCl in the ground water it might be possible to avoid the problem with reduced shell quality. White Leghorn has shown both a significant reduction in shell quality (Pourreza et al., 1994) as well as not being affected (Damron 1998). An explanation for this might be the fact that there are large individual differences in sensitivity towards saline water. Some hens continue to lay eggs with good shell quality when drinking saline water, but increasing numbers are affected as the concentration goes up (Balnave et al., 1989). This shows that it is essential to have a relatively large number of hens used in the study in order to be able to come to any conclusions. The difference between strains opens up for the question; is it possible to breed for a less NaCl sensitive hen?

Several studies have shown that an increased concentration of NaCl in the drinking water causes a reduced CA activity in the shell gland (Yoselewitz & Balnave, 1989a; Dionisiosese & Miyachi, 1992; Balnave & Zhang, 1993). The importance of CA for a normal formation of the eggshell was proven as early as 1945 by Gutowska & Mitchell. Since CA catalyses the hydration of CO_2 to HCO_3^- this may reduce the availability of carbonate ions needed for shell formation. Furthermore, a lowered activity of CA may also lead to a reduction of available Ca^{2+} since the transport of Ca^{2+} through the shell gland mucosa has been shown to be coupled with HCO_3^- (Lundholm, 1990). This could result in a reduced amount of formed calcium carbonate, resulting in a reduced shell quality. Supplying the diet with bicarbonate has proven to increase the shell quality in hens under heat-stress (Balnave & Muheereza, 1997), but if the same goes for hens drinking saline water is unclear. It is possible that the reduction of CA activity would have a negative impact on the transportation of HCO_3^- from the blood to the shell gland, and supplementing the diet with HCO_3^- might therefore be inadequate. The concentration of HCO_3^- has also been shown to be positively correlated with oviposition values. A lower concentration may therefore result in a shortened time for the egg in the shell gland. This would lead to a thinning of the eggshell and eventually resulting in a shell-less egg (Balnave et al. 1989). Exactly how NaCl affects CA is not known, it is suggested that an excess of Cl^- can inhibit the CA activity (Dionisiosese & Miyachi, 1992), but further studies need to be done before a conclusion can be drawn.

So what can be done to decrease these negative effects? It has been established that deleting NaCl from the diet for hens receiving saline drinking water does not improve shell quality (Yoselewitz & Balnave 1989b; Pourreza et al., 1994), due to the difference in biological responses. The response of NaCl consumed through drinking water differs from those obtained when it is included in the diet. But supplementing the diet with Zinc (Moreng et al., 1992; Balnave & Zhang, 1993) has been shown to improve the shell quality. CA is a zinc-dependent enzyme and supplementation of zinc increases the activity of CA, which may lead to a better shell quality. The concentration of CA is not affected and therefore would the most efficient way be to supply zinc while the bird is drinking saline water. Another way that has proven to have beneficial effects on the shell quality is supplying the feed or water with AA (Thornton & Moreng, 1959; Balnave et al., 1991; Balnave & Zhang, 1992). Exactly how AA improves the shell quality in hens under environmental stress is yet not known. It is easy to believe that supplementing the food with Ca^{2+} would improve the shell quality, but NaCl in the drinking water decreases the concentration of CaBP (Balnave & Zhang, 1993). This reduces the amount of Ca^{2+} that can be transported via the blood to the shell gland. Adding calcium to the diet would therefore be of no advantage in this situation.

In conclusion, NaCl is essential for poultry, but concentrations too high received in the drinking water has severe adverse effects on the shell quality in laying hens, possibly through inhibition of CA, an enzyme which is required for normal eggshell production. The exact effect of NaCl on the eggshell formation is yet not fully understood and further research is needed. There are however some methods today that can decrease the negative impact of NaCl on eggshell quality. The best remedy is desalination, but preventative treatments such as supplementing the diet or water with ascorbic acid or zinc have also proven beneficial for the shell quality.

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