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The Effects of in ovo Injection of Ascorbic Acid on Chick Hatchability and Body Weight

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ABSTRACT

The effects of *in ovo* injection of ascorbic acid (AA) on hatchability and chick weight were tested in two trials using a total of 1440 eggs. Varying levels of AA in both free form and salt form were injected into nine different treatments groups on day 18 of incubation, and the results were compared to two non-injected and two water-injected control groups. The treatment groups received either 0.1 ml H_20 with 25 mg AA, 0.2 ml H_20 with 50 mg AA, or 0.3 ml H_20 with 75 mg AA. The pH levels were 2.13 (free form AA) and 7.81 (salt form AA).

The higher AA doses, 0.2 ml H_20 with 50 mg AA and 0.3 ml H_20 with 75 mg AA, demonstrated a significant negative effect on hatchability, independent of pH levels. Chick weight was not significantly affected by AA injections at any level. Though chick weight disparities existed between treatment groups and control groups, these disparities were not indicative of an effect due to AA because of the low hatchability rates for the higher dose-injected groups.

The pH of AA was not determined to be the mechanism affecting either hatchability or chick weight. Rather, some physiological property of AA is most likely causing the decreased hatchability.

INTRODUCTION

Until recently, the supplementation of ascorbic acid (AA) in the diets of poultry has been considered unnecessary since domestic fowl have the innate ability to synthesize AA (Pardue and Thaxton, 1986). Further, under normal conditions, poultry can produce enough AA for optimal functioning of their metabolic processes (Chaudhuri and Chaterjee, 1969; Scott, 1975).

The site of AA production in the domestic fowl is the kidney microsomes. The biochemical pathway through which AA is produced begins when D-glucuronic acid becomes L-gulonic acid. The proceeding steps convert L-gulonic acid into L-gulonolactone, 3-keto-L-gulonolactone, and finally L-ascorbic acid (Kratzer et al., 1996). The synthesis of AA occurs during the fourth day of incubation (Ray, 1934). The rate of AA production in the fowl is 120 μg AA per 1 mg protein per hour *in vitro* (Chaudhuri and Chaterjee, 1969).

The domestic fowl utilizes AA in the biosynthesis of collagen (Gould, 1961; Weiser et al., 1988), vitamin D metabolism (Weiser et al., 1988), and mineral metabolism (Roberson and Edwards, 1994).

However, environmental, nutritional, and pathological stressors have been shown to reduce the bird's ability to synthesize AA (Pardue and Thaxton, 1986). The anti-stress effects of AA have been noted by many (Pardue and Thaxton, 1986; Fletcher and Carson, 1991; Satterlee et al., 1994; Zakaria and Al-Anezi, 1996). Thus, supplementation of AA during incidences of stress could reduce the adverse of stress.

AA has also been demonstrated to play a role in preventing disease and improving immunity. Supplementation of AA during the brooding period of domestic quail significantly lowered mortality rate and improved feed conversion (Wilson, 1989). The addition of AA to

poultry diets has been linked to increased resistance to bacterial infections (Gross, 1988).

Though little research has correlated AA with the chick embryo, it has been suggested that supplementation by egg injection during incubation could improve bone mineralization, decrease bacterial infections, and reduce stress associated with piping and hatching, thus improving hatchability and causing increased chick body weight at hatch (Hagedorn et al., 1992).

This experiment attempted to determine the effects of injecting AA into broiler eggs on day 18 of incubation. Previous studies found beneficial effects when AA was added to the diets of broilers during stressful conditions such as high temperature. During hatch, chicks face multiple stressors including increased heat, higher expended energy levels associated with piping, and adjusting to a new environment that includes handling, vaccination, and transportation soon after hatch. The effects of these stressors reduces the overall performance of chicks, resulting in lower hatchability and body weight, among other factors. Thus, the *in ovo* injection of AA prior to hatch may aid in reducing the stressors, thereby yielding significant benefits for both the chick and the poultry industry.

LITERATURE REVIEW

Stressors and AA

Stress has been defined as a response to certain stimuli perceived to be a threat or a challenge (North, 1984). The hypothalamus registers stress in poultry, receiving impulses through nerves. The hypothalamus transfers the impulses to the adrenal gland, which produces corticosterone, and also produces peptide hormones which stimulate the pituitary gland. The pituitary gland then releases adrenocorticotropic hormone (ACTH). The ACTH stimulates the adrenal glands to release more corticosterone. Thus, the measurement of corticosterone levels present in poultry is used to quantitatively detect the presence of stress.

Environmental, nutritional and pathological stressors can reduce the bird's ability to synthesize AA. Adding AA to the diets of chicks under stress, especially heat stress, reduced adverse responses and increased survivability (Pardue and Thaxton, 1986). Supplementation of AA has caused improvements in egg production, shell quality, and albumen quality in stressed hens (Perek, 1984). Injections of AA at very low levels (3 mg) on day 15 of incubation have been shown to increase hatchability and chick weight (Zakaria and Al-Anezi, 1996).

Effects of Stress

Stress is responded to in three stages: alarm, resistance, and fatigue. Epinephrine and nor-epinephrine control the first stage of alarm, while corticosterone controls the stage of resistance. Corticosterone reduces stress through sustaining gluconeogenesis, the formation of glucose within the body. This insures an abundant supply of energy to help maintain the resistance stage (Pardue and Thaxton, 1984).

Fatigue results only when resistance fails. Fatigue may lead to severe protein

degradation, stunning, and death.

Lesser effects of stress include poor production qualities such as low growth rates and low rates of egg lay.

Role of AA

Previous research has shown that the antioxidant properties of AA limit cellular disruption during times of intense heat stress (Pardue et al., 1985). AA also aids in formation and maintenance of an intercellular substance that binds tissues together. During times of intense heat stress, the small amount of this material present in capillaries leads to ruptures and hemorrhaging in these vessels, the formation of weak bones, atrophy of bone marrow, and anemia (Sackheim and Lehman, 1985).

Under normal conditions, AA is produce in kidney microsomes in poultry, which release AA into the blood to reduce free radicals and prevent subsequent degradation of cellular integrity.

Popular research speculated that AA could play an important role in disease resistance, resulting in positive effects ranging from alleviating the common cold to curing cancer. However, research has not been able to validate these claims (Thomas and Holt, 1978).

Incubation and AA

During incubation, several stressors exist which may have a detrimental effect on the developing embryo. These include an increase in heart rate (Cain et al., 1967), and a 1.5° C rise above incubator temperature inside of the chorioallantoic membrane due to increased metabolic heat (Sotherland et al., 1987). Research has also indicated beneficial effects from lowering incubation temperature during the latter part of incubation (Tullet, 1990) and cooling broiler

breeder eggs to 22° C on day 16 of incubation (Sarpong and Reinhart, 1985).

MATERIALS AND METHODS

Two trials were run to determine the effect of the *in ovo* injection of ascorbic acid on chick hatchability and body weight. The first trial dealt with the effects of free form AA on these factors, while the second trial tried to determine whether pH of AA affected either factor. In both experiments 720 eggs were utilized, for a total sample size of 1440 eggs. All eggs were obtained from Sanderson Farm's commercial hatchery, and stored at 18° C for no more than four days before incubation. Eggs were randomly assigned to treatment groups.

Setting

Each egg was individually weighed. Weights were written on the small end of the eggs in pencil. All eggs were then placed into a Robbins model 14I setter. Temperature was maintained within the setter at 37.5° C. Incubation proceeded for 18 days.

Candling

During incubation, the eggs were candled three times to determine both fertility and viability. On day 7, a small hand candler was used to determine fertility. Infertile eggs were discarded, as well as early mortalities. Fertile, viable eggs were returned to the incubator. Viability was reassessed on days 14 and 18.

Treatments

- Trial One

  Trial one consisted of a non-injected control, a sham group injected with 0.3 ml sterile, deionized water, and three AA treatment groups. The AA groups were injected with 0.1 ml, 0.2 ml, or 0.3 ml of a 25 mg/0.1 ml solution of AA. The dose levels were 25 mg, 50 mg, and 75 mg AA, respectively.

- Trial Two

Trial two consisted of a non-injected control group, a sham group injected with 0.3mL of sterile, deionized water, and six AA treatment groups. The AA groups were injected with 0.1 ml, 0.2 ml, or 0.3 ml of a 25 mg/0.1 ml solution of AA. The dose levels were 25 mg, 50 mg, and 75 mg AA, respectively. Three of the groups received free form AA, replicating trial one, and three of the groups received a salt form.

Preparation of Solution

L-AA was obtained from Sigma Chemical Company, St. Louis, Mo. To prepare solutions for trial one, 12.5 g of AA was dissolved in 50 ml of sterile, deionized water, and divided into three equal portions in three 80 ml beakers. A Mettler model AE 100 scientific scale was used to measure all allotments of AA. A Sargent-Welch model S-76491 magnetic stirrer, was used to dissolve the AA into solution. The resulting concentration for each of the three solutions was 25 mg/0.1 ml.

To obtain solutions for the free form groups in trial two, 12.5 g of free form AA was dissolved in 50 ml of sterile, deionized water in a manner similar to trial one. The resulting concentration for each of the three solutions was 25 mg/0.1 ml. The pH of this solution was 2.13. For the salt form groups, 12.5 g of AA sodium salt was dissolved in 50 ml of sterile, deionized water. The resulting concentration was 25 mg/0.1 ml. The pH of this solution was 7.81.

Injections

All injected groups were subjected to the following procedure:

Eggs were removed from the incubator on day 18 of incubation. All eggs were candled for the final time. A small hole was then etched into the large end of the egg directly above the air cell using an ordinary engraving tool. Then, the eggs received the appropriate treatment dosage through a one cc syringe equipped with an 18 gauge needle. The site of injection was just beyond the air cell into the albumen of the egg.

Upon completion of the injections, eggs in trial one were sealed with melted paraplast and immediately placed into a Robbins H10 hatcher. Eggs in trial two were unsealed. The eggs were maintained at 37.5° C for three days in the hatcher. On day 21, all hatched chicks, unhatched eggs and pipped eggs were removed from the hatcher.

Data Collection

Data were recorded on hatchability and chick weight. Fertile hatchability was calculated. Any egg found unhatched was broken out to ensure that at the time of injection the embryo was indeed viable. Any embryo not in a normal hatch position, with its head under its right wing, was determined to have died prior to injection and therefore removed from the experiment. Each chick was weighed individually.

Statistical Analysis

Statistical analysis was performed by using the Statistical Analysis System (SAS, Barr et al., 1985). Hatch data, a categorical response, were subjected to the chi-square test in the first trial and analysis of variance (ANOVA) in the second trial. Data collected on chick weight were analyzed by ANOVA. Means were separated by Duncan's multiple range test. A probability

level of 0.05 was used in determining significance.

RESULTS AND DISCUSSION

This experiment tested the effects of the *in ovo* injection of AA on both hatchability and chick weight. Previous experiments indicated that low doses of AA (3mg) during the latter period of incubation caused a significant increase in body weight and also improved hatchability (Zakaria and Al-Anezi, 1996).

The lowest level AA injected group, 0.1 ml H_20 with 25 mg AA, had an average hatchability comparable to both control groups (Table 1). However, the higher AA treatment groups had significantly lower hatchability rates than any of the other groups. At levels of 0.2 ml H_20 with 50 mg AA and 0.3 ml H_20 with 75 mg AA, the hatchability was 45.5% and 0.0%, respectively.

Each of the treatment groups was statistically identical to each other when chick weight was considered (Table 2). Data from the group receiving the highest level of AA injections, 0.3 ml H_20 with 75 mg AA, were excluded because no chicks hatched, as shown in Table 1.

The groups injected with AA in trial 2, both the salt and free from, had a significantly lower hatchability than either of the control groups (Table 3). Only the low dose of the free form had a hatchability statistically similar to the control groups. These results are consistent with previous findings in 1992 by Palmer, that low level injections of AA in free or salt form have no significant effect on hatchability.

Because the pH varied significantly between the salt form (pH=7.8) and the free form (pH=2.13), yet the hatchability was consistently poor regardless of AA type, it is inferred that AA, not pH, negatively affects hatchability through some mechanism. This coincides with previous research that a salt form of AA is significantly different from the free form of AA in the

Table 1. Hatchability of Broiler Breeder Eggs Given Ascorbic Acid Injections on Day 18 of Incubation (Trial 1).

Treatment	**Hatchability (%)**
non-injected	87.8[a]
0.3 ml sterile H_20	75.1[a]
0.1 ml H_20 with 25 mg AA	79.3[a]
0.2 ml H_20 with 50 mg AA	45.5[b]
0.3 ml H_20 with 75 mg AA	0.0
	p=.004

a,b means with different letters are significantly different ($p<.05$)

Table 2. Chick Weights of Broiler Breeder Eggs Given Ascorbic Acid Injections on Day 18 of Incubation (Trial 1).

Treatment	**Chick Weight (g)**
non-injected	44±4[a]
0.3 ml sterile H_20	44±3[a]
0.1 ml H_20 with 25 mg AA	44±2[a]
0.2 ml H_20 with 50 mg AA	43±3[a]
0.3 ml H_20 with 75 mg AA	-----

p=.56

hatchability rate, and that a salt form may even have a deleterious effect if injected at higher levels (Palmer, 1992).

The hatchability rates also coincide with Zakaria and Al-Anezi's findings in 1996 that a 12 mg dose injected on day 15 had adverse effects, including decreased embryo weight and low hatchability. There have been no studies done on the effects of high dose AA injections during the latter stages of embryonic development. However, it may be inferred that the low hatchability rate among AA injected groups may be attributed to the findings that an increasing concentration of AA is accompanied by a selective toxic action on the pancreatic beta cells (Meglasson and Hazelwood, 1982), a death of resting cells, and the reversal of differentiation (Iyengar and Lal, 1982), or the death of mesenchymal cells, and with those deaths a decrease in mineralization (Boskey et al., 1991).

Those groups receiving higher doses of AA in the free form were also noted to have hemorrhaging. This was consistent with a previous study that found severe body hemorrhages at an injection level of 12 mg AA (Zakaria and Al-Anezi, 1996). No further explanation of the hemorrhaging was given.

The only two treatments that are significantly different from the control groups are the mid-level free from AA injection and the high-level salt form AA groups (Table 4). The mid-level free form AA injected group has a significantly lower average chick weight than the control groups. The high-level salt form AA injected group had a significantly higher average chick weight when compared to the control groups.

However, due to the low hatch rates of these groups (21.7% for the high-level AA injected group and 13.3% for the mid-level free form AA injected group) those chicks that did

hatch may have had a predisposed heightened chance of hatching due to their size. Those that hatched were probably the largest of the chicks receiving the injections, and could therefore survive a larger injection dose than smaller chicks.

Table 3. Hatchability of Broiler Breeder Eggs Given Ascorbic Acid Injections on Day 18 of Incubation (Trial 2).

Treatment	**Hatchability (%)**
non-injected	91.5[a]
0.3 ml sterile H_20	95.0[a]
0.1 ml H_20 with 25 mg AA (free form)	83.1[a]
0.2 ml H_20 with 50 mg AA (free form)	13.3[c]
0.3 ml H_20 with 75 mg AA (free form)	10.0[c]
0.1 ml H_20 with 25 mg AA (salt)	53.3[b]
0.2 ml H_20 with 50 mg AA (salt)	8.6[c]
0.3 ml H_20 with 75 mg AA (salt)	21.7[c]

p=.0001

a, b, c means with different letters are significantly different ($p<.05$)

Table 4. Chick Weights of Broiler Breeder Eggs Given Ascorbic Acid Injections on Day 18 of Incubation (Trial 2).

Treatment	**Chick Weight (g)**
non-injected	44.5 ± 3.7^{bc}
0.3 ml sterile H_20	44.4 ± 3.7^{bc}
0.1 ml H_20 with 25 mg AA (free form)	43.7 ± 3.4^{c}
0.2 ml H_20 with 50 mg AA (free form)	40.9 ± 6.6^{d}
0.3 ml H_20 with 75 mg AA (free form)	43.7 ± 3.5^{c}
0.1 ml H_20 with 25 mg AA (salt)	43.8 ± 3.4^{c}
0.2 ml H_20 with 50 mg AA (salt)	46.3 ± 5.1^{ab}
0.3 ml H_20 with 75 mg AA (salt)	48.2 ± 2.3^{a}

p=.0002

a, b, c, d means with different letters are significantly different ($p<.05$)

CONCLUSION

The purpose of the experiment was to determine whether AA injections on day 18 on incubation would affect hatchability and/or chick weight. Further, in trial two, the effect of AA's pH was assessed to determine whether the acidic effects of AA affected the outcomes of hatchability and/or chick weight.

The results concluded that low level AA injections of 0.1 ml H_20 with 25 mg AA do not significantly affect hatchability. Hatchability rates for this level of AA injection are consistent with those of control groups.

However, higher doses, 0.2 ml H_20 with 50 mg AA and 0.3 ml H_20 with 75 mg AA, have a significant negative effect on hatchability. Hatchability scores for the mid-level AA injected groups were 45.5% in trial one, 13.3% (free form) and 8.6% (salt form) in trial two. Hatchability scores for the highest level AA injected groups were 0.0% in trial one, 10.0% (free form) and 21.7% (salt form) in trial two. Therefore, there is a demonstrated inverse relationship between level of AA injection and hatchability, independent of pH levels.

The results concluded that chick weight was not significantly affected by AA injections at any level. Though chick weight disparities exist between treatment groups and control groups, these disparities are not indicative of an effect due to AA. Because of the low hatchability rates for the groups determined to be significantly different from control groups, the number of chicks in the sample was too small; thus, no valid estimation of AA's effects on chick weight can be delineated.

The most interesting finding of this experiment is that pH of AA is not the mechanism

affecting either hatchability or chick weight. It is currently theorized that any detrimental effects of AA on animal species is due to the acidic effects of AA. However, trail two of this experiment demonstrated no significant differences attributable to acidic effects. The salt form (pH=7.81) and the free form (pH=2.13) affected both hatchability and chick weight in the same direction and intensity.

Thus, the mechanism whereby AA affects hatchability lies in the innate physiological properties of AA, not in an acidic imbalance/overdose in the embryo. Further research is needed to further attribute the affects on hatchability caused by AA.

REFERENCES

Barr, A.J., J.H. Goodnight, J.P. Sall, W.H. Blair, and D.M. Chilko, 1985. SAS User's Guide. SAS Inst., Cary, NC.

Boskey, A.L., D. Stiner, S.B. Doty, and I. Binderman, 1991. Requirement of vitamin C for cartilage calcification in a differentiating chick limb-bud mesenchymal cell. Culture. Bone 12:277-282.

Cain, J.R., U.K. Abbot, and V.L. Rogallo, 1967. Heart rate of developing chick embryo. Proc. Soc. Exp. Biol. Med. 127:507-510.

Chaudhuri, C.R. and I.B. Chaterjee, 1969. L-ascorbic acid synthesis in birds: Phylogenetic trend. Science. 164:435-436.

Fletcher, D.L. and J.A. Carson, 1991. Influence of ascorbic acid on broiler shrink and processing yields. Poultry Sci. 70:2191-2196.

Gould, B.S., 1961. Ascorbic acid-independent and ascorbic acid-dependent collagen forming mechanisms. Ann. N. Y. Acad. Sci. 92:168-174.

Gross, W.B., 1988. Effect of environmental stress on the responses of ascorbic acid-treated chickens to Escherichia coli challenged infection. Avian Dis. 32:432-436.

Hagedorn, T.A., D.R. Ingram, R.A. Phillips, S. Duggan, and A. Melsheimer, 1992. Effects of ascorbic acid on embryonic development. Poultry Sci. (Suppl. 1) 71:7.

Iyengar, B. and S.K. Lal, 1982. The effect of ascorbic acid on a proliferating cell system. Int. J. Tiss. Reac. 4:265-268.

Kratzer, F.W., H.J. Almquist, and P. Vohra, 1996. Effect of diet on growth and plasma ascorbic acid in chicks. Poultry Sci. 75:82-89.

Meglasson, M.D., and R.L. Hazelwood, 1982. Ascorbic diabetogenesis in the domestic fowl. Gen. Comp. Endocrinol. 47:205-212.

North, M.O., 1984. Commercial chicken production manual, third edition. The Avi Publishing Co., Inc. Westport, CT.

Palmer, L.A., 1992. The relationship of AA injection to hatchability parameters of broiler breeder eggs. MS Thesis.

Pardue, S.L., and J.P. Thaxton, 1986. Ascorbic acid in poultry: A review. World's Poultry Science J. 42:107-123.

Pardue, S.L., and J.P. Thaxton, 1984. Evidence for amelioration of steroid-mediated immunosuppression by ascorbic acid. Poultry Sci. 63:1262-1268.

Pardue, S.L., J.P. Thaxton, and J. Brake, 1985. Role of ascorbic acid in chicks exposed to high environmental temperature. J. of Appl. Phys. 58:1511.

Perek, M., 1984. Ascorbic acid and the endocrine system with emphasis on the stress problem in poultry. In Proceedings of Workshop on Ascorbic Acid. I. Wegger, F., J. Tagwerker, and J. Mousstgaard, Eds., The Royal Danish Agricul. Soc., Copenhagen.

Ray, S.N., 1934. A note on the presence of vitamin C in the chick embryo. Biochemical Journal. 28:189.

Roberson, K.D. and H.M. Edwards, 1994. Effects of ascorbic acid and 1,25-dihydroxycholecalciferol on alkaline phosphatase and tibial dyschondroplasia in broiler chickens. Br. Poultry Sci. 35:763-773.

Sackheim, G. I. and D.D. Lehman, 1985. Chemistry for the Health Sciences. Macmillan Publishing Company. New York, NY.

Sarpong, S., and B.S. Reinhart, 1985. Broiler hatching stress and subsequent growout performance. Poultry Sci. 64:232-234.

Satterlee. D.G., R.B. Jones and F.H. Ryder, 1994. Effects of ascobyl-2-Polyphosphate on adrenocortical activation and fear related behavior in broiler chickens. Poultry Sci. 73:194-201.

Scott, M.L., 1975. Environmental influences on ascorbic acid requirements in animal. Ann. New York Acad. Sci. 258:151-155.

Sotherland, P.R., J.R. Spotila, and C.V. Paganelli, 1987. Avian eggs: barriers to the exchange of heat and mass. J. Exp. Zool. Suppl. 1:81-86.

Thomas, W.R., and P.G. Holt, 1978. Vitamin C and immunity: An assessment of the evidence. Clin. Exp. Immunol. 32:370-379.

Tullet, S.G., 1990. Science and art of incubation. Poultry Sci. 69:1-15.

Weiser, H., M. Schlachter and H. Bachman, 1988. The importance of vitamin C for hydroxylation of vitamin D3 to la25(OH)D3 and of 45R.25(OH)2D3 to a more active metabolite. Pages 644-653 in: Proceedings of the Seventh Workshop on Vitamin D, Rancho Mirage, CA, Walter de Gruyter, Berlin, Germany.

Wilson, H.R., 1989. Research note: Mortality in Bob White quail as affected by supplemental

ascorbic acid. Poultry Sci. 68:1418-1420.

Zakaria, A.K. and M.A. Al-Anezi, 1996. Effect of ascorbic acid and cooling during egg incubation on hatchability, culling, mortality, and the embryo weights of broiler chickens. Poultry Sci. 75:1204-1209.