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A STUDY ON THE EMBRYOTOXIC IMPACT OF PURE PROPIONIC ACID ADMINISTERED VIA IN OVO ROUTE IN QUAIL EGGS

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ABSTRACT

The aim of this study was to investigate the effects of in ovo injection of propionic acid on hatchability and chick viability in quail eggs. A total of 160 fertilized quail eggs were randomly divided into four groups (n=40 per group). The control group received no treatment; the perforation group had only a hole made and sealed; the treatment group received 0.02 ml of pure propionic acid on day 15 of incubation; and the sham group received 0.02 ml of distilled water via in ovo injection. The injection was performed into the embryonic cavity through the air sac under sterile conditions. Hatchability was significantly lower in the propionic acid group compared to the control ($P<0.001$), while the perforation and sham groups showed similar hatchability to the control. No significant differences were observed among the groups regarding early embryonic mortality ($P = 0.788$). However, late embryonic mortality was significantly higher in the propionic acid group ($P<0.001$), while the perforation and sham groups were similar to the control. No significant differences were found among the groups in terms of chick yield or egg weight loss ($P>0.05$). As a result, it was observed that pure propionic acid had a toxic effect by increasing embryonic mortality and significantly increased the late mortality rate of quail.

Keywords: Quail, egg, in ovo, propionic acid

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INTRODUCTION

In poultry farming, feeding chicks a balanced and highly digestible ration as soon as possible after hatching has a well-documented positive impact on growth performance, health, and overall productivity during subsequent rearing periods. However, since eggs cannot absorb external nutrients during incubation, alternative strategies have been explored to enhance embryonic nutrition and early development. One such approach is **in-ovo technology**, which involves the direct injection of substances into the egg during incubation to increase nutrient availability and improve chick quality (1).

In-ovo technology was first introduced in the late 1970s, and by 1995, the development of an automated injection system made its application more feasible on a commercial scale (2). Initially, this technique was applied primarily for vaccination purposes, enabling large-scale immunization of embryos against common poultry diseases. Over time, however, its potential has expanded to include the delivery of nutrients, probiotics, prebiotics, amino acids, vitamins, and even bioactive compounds aimed at improving gut development, immunity, and post-hatch performance (3, 4).

Research has shown that in-ovo feeding of specific nutrients can enhance intestinal maturation, increase villi surface area, and support the establishment of a healthier gut microbiota in newly hatched chicks (5). Moreover, supplementation with certain amino acids such as threonine and glutamine has been linked to improved immune responses and growth rates, while carbohydrate and protein solutions have been reported to increase body weight at hatch. The final few days before hatching and the first days after hatching are considered the most critical period in chick development. During this time, rapid growth and differentiation occur, with intestinal development progressing at its fastest rate (4). However, under commercial conditions, newly hatched chicks often experience a fasting period of approximately 48–72 hours due to the time required for processing, vaccination, sexing, transport, and placement in production facilities. This delay in feed and water access can impair gastrointestinal tract maturation, retard immune system stimulation, and ultimately reduce chick viability and growth potential.

To mitigate these negative effects, **in-ovo feeding** has been proposed as a strategy to provide nutrients or bioactive supplements directly to the embryo before hatch. By supplying targeted compounds such as carbohydrates, amino acids, vitamins, minerals, or immune-stimulating agents, in-ovo feeding can enhance embryonic nutrient availability, support earlier gut maturation, and stimulate immune development. As a result, hatched chicks may be better equipped to withstand the detrimental effects of post-hatch feed deprivation (6).

Several studies have demonstrated the benefits of in-ovo feeding on multiple physiological systems. For instance, in-ovo feeding has been reported to promote skeletal development by improving bone mineralization, to enhance immune function through earlier lymphoid organ

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development and antibody responses, and to accelerate digestive system maturation by increasing villi height, crypt depth, and enzymatic activity in the small intestine (7, 8). Collectively, these improvements highlight in-ovo feeding as a promising tool for optimizing chick health and performance under commercial production conditions. In addition, plant-derived extracts and organic acids have been investigated for their antimicrobial, immunomodulatory, and growth-promoting properties (6).

Organic acids, widely recognized as effective feed additives, are naturally occurring compounds produced through microbial fermentation of carbohydrates. Their primary role in animal nutrition lies in modulating the gastrointestinal microflora by promoting the proliferation of beneficial microorganisms while simultaneously inhibiting the growth of pathogenic species (9, 10). By lowering the gastric pH, organic acids create an unfavorable environment for harmful bacteria, thereby reducing the passage of pathogens from the stomach to the intestines and supporting a healthier microbial balance (10).

Among these compounds, **propionic acid** has received particular attention due to its broad-spectrum antimycotic and antibacterial properties. Its antimicrobial activity is mediated through multiple mechanisms, including the disruption of bacterial enzyme complexes, impairment of DNA replication, and destabilization of cell membranes, ultimately leading to bacterial death (11). These characteristics make propionic acid not only a valuable preservative but also a potential enhancer of animal health and performance. Importantly, studies have reported that the supplementation of organic acids in feed or drinking water does not pose risks to humans or the environment, supporting their safe use in modern animal production systems (12).

The aim of this study was to investigate the effects of in ovo injection of propionic acid on hatchability and chick viability in quail eggs.

MATERIALS AND METHODS

The study was conducted at the Balikesir University Faculty of Veterinary Medicine Practice Farm. The necessary project-based permits for the study were obtained from the Balikesir Provincial Directorate of Agriculture and Forestry. Additionally, an application was made to the Balıkesir University Animal Experiments Local Ethics Committee and approval was received (Approval number: 2024/4-2).

Eggs and experimental design

A power analysis was conducted to determine the minimum sample size required for the study. The analysis was based on a Type I error (α) of 0.05 and a statistical power ($1-\beta$) of 0.90. The effect size was assumed to be moderate ($f = 0.4$), based on data from similar studies in the literature. The calculation was performed using one-way ANOVA assumptions. The analysis indicated that

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at least 7 samples per group were required. To increase the reliability of the findings and account for potential losses during incubation, a total of 160 fertilized quail eggs (40 per group) were used in the experiment. Eggs were divided into four groups, each containing 40 eggs: the control group (no treatment), the perforation group (only holes were drilled and closed), the propionic acid group (0.02 ml propionic acid ≥99% purity, Sigma-Aldrich, St. Louis, MO, USA on day 15 of incubation), and the negative control group (0.02 ml distilled water). In ovo applications were performed by injecting the eggs into the embryonic cavity through a hole in the air sac under appropriate sterilization conditions.

Determination of Hatching Characteristics

Hatching efficiency (%): (Number of chicks hatched / number of eggs laid) x 100,

Hatchability (%): (Number of chicks hatched / total number of fertile eggs) x 100,

Early embryonic mortality rate (%): (Number of chicks dying in the early period / total number of fertile eggs) x 100,

Mid-term embryonic mortality rate (%): (Number of chicks dying in the mid-term / total number of fertile eggs) x 100,

Late-term embryonic mortality rate (%): (Number of chicks dying in the late period / total number of fertile eggs) x 100,

Chick yield (%): (Weight of hatched chicks (g) / Weight of eggs laid (g)) x 100,

Moisture loss (Egg weight loss on day 15 of incubation) (%): (Weight of initial eggs (g) / (initial egg weight – The weight of eggs on the 15th day) was calculated according to the x100 formula (13).

Statistical Analysis

All analyses were conducted using the IBM®SPSS 20 package program. Hatchability and mortality rates were compared using chi-square tests of independence, while moisture loss and chick turnover rates were compared using one-way analysis of variance (One-Way ANOVA). Data are presented as mean and standard error. Statistical significance was accepted at $P<0.05$.

RESULT

Table 1 shows the hatchability of quail eggs. According to the study results, the lowest hatchability of all groups was observed in the propionic acid group. Hatching ability in the sham and perforation groups was found to be similar to the control group ($P>0.05$).

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Table 1: The hatching efficiency of the groups

Groups	Hatching efficiency (%)	df	X^2	P-value
Control	88.00[a]	3	130.848	<0.001
Propionic acid	2.08[b]			
Sham	89.79[a]			
Perforation	92.00[a]			

[a,b]: Different superscripts indicate statistical differences between groups. df: degree of freedom, X^2: Table chi-square value

Table 2 presents the early and late embryonic mortality rates for the groups. No mid-stage or subshell mortality was observed in the study. Differences between the groups in terms of early embryonic mortality were found to be insignificant (P>0.05). In terms of young embryonic mortality, the highest embryonic mortality was observed in the propionic acid group compared to the control group (P<0.001), while the sham and perforation groups were similar to the control group. Late embryonic mortality observed on the day of hatching is demonstrated in Figure 1.

Table 2: Embryonic mortality of the groups

Groups	Early (%)	df	X^2	P-value
Control	3.92	3	1.053	0.788
Propionic acid	6.25			
Sham	4.08			
Perforation	2.00			
	Late-term (%)	df	X^2	P-value
Control	7.84[a]	3	60.023	<0.001
Propionic acid	91.66[b]			
Sham	6.12[a]			
Perforation	6.00[a]			

[a,b]: Different superscripts indicate statistical differences between groups. df: degree of freedom, X^2: Table chi-square value

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Figure 1: An embryonic death of late-term (Original)

The chick yield is given in Table 3. The difference between the groups in terms of chick yield was not statistically significant (P>0.05).

Table 3: Chick yield of the groups

Groups	Chick yield (%)	F value	P-value
Control	65.50±1.22	0.788	0.457
Propionic acid	67.32±1.28		
Sham	66.31±1.23		
Perforation	66.94±1.20		

Data are presented as mean and standard error. F value: Table F value of ANOVA test

Moisture loss values for the groups are given in Table 4. No statistically significant difference was found between the groups in terms of moisture loss (P>0.05).

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Table 4: Moisture loss of the groups

Groups	Moisture loss (%)	F value	P-value
Control	6.84±0.20	1.369	0.254
Propionic acid	7.09±0.28		
Sham	7.27±0.23		
Perforation	6.60±0.23		

Data are presented as mean and standard error. F value: Table F value of ANOVA test

DISCUSSION

Propionic acid is widely used in the food and feed industries for its antimicrobial effects (14). However, the high concentration of pure propionic acid used in this study may have had a toxic effect on the embryo. Some studies in the literature emphasize that the dose and method of administration of organic acids in in ovo use are critical for embryo development (15). It is well established that, particularly during the late stages of embryonic development, sensitive tissues are more susceptible to chemical agents (16). Examination of the underlying mechanisms suggests that organic acids may adversely affect embryonic development by altering intracellular pH, disrupting ion homeostasis, compromising cell membrane integrity, and impairing mitochondrial function (17). In in ovo applications, the injection dose has been reported to have a critical effect on embryo development in several studies (4, 18). For example, formic acid administration from 0.03 mM to 32 mM increased embryonic mortality rates and demonstrated toxicity; some studies also indicate that co-administration of protective agents (e.g., kinetin) is beneficial to reduce toxicity (19). The results indicate that hatchability was significantly reduced in the propionic acid-treated group compared to the control group, accompanied by a marked increase in late-stage embryonic mortality. The toxic effects of propionic acid have also been demonstrated in various model systems. In a study conducted in Drosophila melanogaster larvae, propionic acid at concentrations of 5–10 mM caused dose-dependent genotoxic effects, oxidative stress, and tissue damage. This finding suggests that similar pathological mechanisms may be at play in hatching embryos at high doses (20). In contrast, these effects were not observed in the perforation-only group or the distilled water-treated group, with both groups achieving similar results to the control group. This finding suggests that neither the injection procedure itself nor the physical perforation had a significant negative impact on embryo viability. In this study, no significant difference was found between the groups in terms of early embryonic mortality. This suggests that the effects of propionic acid occur at later stages of embryo development. Indeed, late-term embryonic mortality was significantly higher in the propionic acid group. These findings suggest that the pure propionic acid used, in addition to its direct toxic effects, may increase the risk of mortality by interfering

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with embryonic development. In in ovo applications of citric acid and lactic acid, low doses did not negatively affect embryonic development, whereas higher doses were associated with increased late-term mortality (16). These findings suggest that embryonic sensitivity is dependent on the developmental stage, and that both the type and concentration of the administered compound can significantly influence the outcomes. The late embryogenesis period, during which organs and metabolic systems undergo functional maturation, is considered particularly vulnerable to environmental stressors and chemical agents (6). In this stage, tissues such as the liver, kidneys, and muscles experience rapid cellular differentiation and heightened metabolic activity, making them especially susceptible to the pH alterations and oxidative stress induced by organic acids, which can lead to fatal consequences for the embryo. No significant differences were found between the groups in parameters such as chick yield and intra-egg moisture loss. This finding suggests that propionic acid does not directly affect water loss during incubation or post-hatch live weight conversion. Intra-egg moisture loss is a critical factor for hatching success and is generally shaped by the permeability of the egg shell, the relative humidity in the incubation environment and the metabolic activity of the embryo (21). In this study, propionic acid application did not significantly affect eggshell water vapor permeability or embryonic metabolism as reflected by moisture loss. This indicates that the increased late-term mortality observed in the propionic acid group was not associated with alterations in water regulation or gas exchange across the eggshell, but was more likely the result of direct toxic effects on developing tissues. Similarly, the chick yield (the ratio of hatched chick weight to egg weight) remained at similar levels regardless of the treatments applied. The literature reports that chick conversion rate is significantly affected by environmental factors such as egg weight, incubation temperature, and humidity, but in ovo treatments generally have limited effects on this parameter (22, 23). From this perspective, it can be said that the toxic effects of propionic acid are concentrated on embryonic viability and are not significantly reflected in embryonic water loss dynamics during incubation or post-hatch chick body weight. On the other hand, the preservation of the chick transformation rate suggests that, when the embryo survives, basic metabolic aspects of development are largely maintained. This suggests that propionic acid may have a selective effect on the embryo, resulting in either severe toxicity and death or relatively preservation of growth parameters in surviving embryos. The most significant limitation of this study is that propionic acid was applied only in its pure form. Future studies are recommended to use different dilution ratios to reduce the toxic effects on the embryo and to investigate its potential beneficial effects.

CONCLUSION

This study demonstrates that in ovo administration of pure propionic acid may have adverse effects on quail embryos. Careful optimization of the application method, dosage, and timing is crucial for the safe and effective use of such organic acids in in ovo applications. The present study

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provides important insights into the potential of in ovo propionic acid application; however, several limitations should be acknowledged, which may guide future research directions. First, testing a wider range of doses or diluted concentrations would enable clearer determination of safe application thresholds. Second, extending the evaluation beyond hatchability and embryonic mortality to include post-hatch growth performance, immune response, and physiological parameters of the chicks would provide a more comprehensive understanding of the effects. Third, since embryonic toxicity was observed, the use of histopathological examinations or molecular markers could help to elucidate the underlying mechanisms of mortality. Fourth, long-term monitoring of chick performance would enhance the practical significance of the findings for poultry production. Finally, validation of the results across different poultry species, such as chickens or turkeys, would improve the generalizability and industrial applicability of the outcomes.

Conflict of Interest

The authors declare that they have no conflict of interest.

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