EFFECT OF *IN OVO* BETAINE-HCL SUPPLEMENTATION ON HATCHABILITY, CHICK QUALITY AND ORGAN DEVELOPMENT OF BROILER CHICKS

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Abstract
Two trials were carried out to assess the effect of in-ovo supplementation of Betaine-HCl on the hatchability, chick quality, performance and organ development of boiler chicks. Trial 1 was carried out using 160 Marshall broiler chicks at 18 day of incubation. Treatments included: un-injected control, 0.9% saline, 0.9% saline + 50mg of Betaine-HCl, 0.9% saline + 100mg Betaine-HCl and 0.9% saline +150mg Betaine-HCl. Trial 2 was carried out using 266 Marshall broiler chicks at 18 day of incubation. Treatments included: un-injected control, 0.9% saline, 0.9% saline + 50mg Betaine-HCl, 0.45% saline + 100mg Betaine-HCl and 0.23% saline + 150mg Betaine-HCl. Parameters observed were hatchability, chick quality (length) and chick weight. In trial 2 additional parameters observed were weight gain as well as relative organ weights. In-ovo Betaine-HCl supplementation was observed to influence various parameters observed ($p<0.05$) in both trials. Liver and pectoral muscle weights weights were also significantly ($p<0.05$) influenced by Betaine-HCl supplementation in-ovo.

Introduction

Glucose is the predominant energy source during the first week of embryonic growth (Moran, 2007), whereas a large portion of the carbohydrate glucose is utilized in the first week of incubation. Glycogen stored in the liver and muscles of embryos is the main energy source during the hatching process (Moran, 2007). However at this stage of incubation, glycogen is utilized in greater amount in order to meet the high energy demand of the hatching process, and consequently glycogen reserves in the embryo are significantly depleted at the end of incubation. Weight loss between hatching and removal from the hatcher is approximately 0.18g/h while fasting (Halevy *et al.*, 2000). Several alternatives have been proposed to improve performance during this initial phase such as *in-ovo* feeding (Uni and Ferket, 2004). This method is based on the fact that neonatal birds naturally consume the embryonic amniotic fluid towards hatch (Romanoff, 1960). Therefore, addition of a nutrient solution to the embryonic amniotic fluid delivers essential nutrients into the embryo intestine. Betaine is distributed widely in animals, plants, and micro-organisms and rich dietary sources include seafood, especially marine invertebrates (1%); wheat germ or bran (1%); and spinach (0.7%). In addition to dietary sources, betaine can be produced through the irreversible oxidation of choline via choline dehydrogenase and betaine aldehyde dehydrogenase. As an osmolyte, betaine protects cells by regulating cell volume, stabilizing proteins, and enzymes from environmental stress (Craig, 2004). Moreover, betaine has been discussed for its role in saving methionine and choline (Fernandez-Figares *et al.*, 2002), making methionine more available for protein synthesis, and making choline more available for lipid metabolism. Thus this study proposed that *in-ovo* supplementation of betaine in hydrochloride form could improve energy storage, consequent hatchability, chick quality and organ development.

Materials and methods

A total of 160 fertile 18days incubated eggs of comparable weight produced by a flock of 45-week-old hens from a meat-type breeder (Marshall), were used to observe the effect of combination of Betaine-HCl and 0.9% saline on hatchability and chick quality. The eggs were examined and selected after candling. The *in-ovo* treatments were two control treatments: non-injected (negative control, NC), and injected with 0.9% sterilised saline (positive control, PC) and three other treatments in which sterile saline containing dissolved quantities of 50, 100 or 150mg of Betaine-HCl was injected. The wide end of the eggshell was disinfected with 70% isopropyl alcohol where-upon an injection hole was pierced. The 1mL of solution was injected into the amniotic sac. After injection, the hole was and the eggs were returned to the incubators. Percent hatchability was calculated on the basis of the number of hatched chicks as a percentage of set eggs. At the end of incubation, chicks were observed and hatching weight was recorded. Measurements were made of hatchability, chick weight and chick quality (chick length and rectal temperature). In the second trial to assess *in-ovo* supplementation of Betaine-HCl and varying saline concentrations,

a total of 266 eggs were evenly assigned to hatching trays. *In-ovo* procedure carried out as described for experiment 1 and all parameters observed in experiment 1 were repeated. At the end of the incubation period, six chicks were randomly selected from each experimental treatment. The liver, pectoral muscle and yolk sac were collected and weighed and calculated as a percentage of the live weight. Data from trials 1 and 2 were analyzed separately using one-way anova and means separation carried out were significant (SPSS, 2008)

Results and Discussion

It was observed that using 0.9% saline solution as buffer at 100mg and 150mg greatly reduc hatchability (Table 1). Kadam *et al.* (2013) als observed that *in-ovo* betaine supplementation did improve hatch weight at 100mg compared with the un-injected control, though other betaine levels were significantly lower. This could be as a result of the fact that in that study Milli Q water was used as the buffer not saline. Even though the osmolarity for both solution were within the range described by Uni and Ferket (2003) as the acceptable range, though they stated their range for osmolality. The negative effect could be as a result in the difference between osmolarity and osmolality, since osmolality takes into consideration practical interactions between solutes. When this was corrected in the trial 2, by varying the saline concentrations, there was marked improvement in hatchability. Betaine-HCl supplementation at 150mg had the highest values, while the un-injected control had the lowest values. Betaine as an osmolyte has been effective as an osmolyte, by protecting cells from stress (Craig, 2004), even though the higher osmolarity in trial 1 could not be ameliorated by the presence of Betaine-HCl. Also since betaine is an ideal methyl donor, it would be required in methylation reactions for methyl donation into the gluconeogenic pathway; which is a major pathway at hatching. This was reflected in the hatching weights of the chicks in both trials. There was significant effect of supplementation observed in trial 1with Betaine-HCl supplementation having higher values compared with the controls, with weight differences as much as 5% increase. There was no difference observed in trial 2 but values still showed numerically higher weights compared with the un-injected controls.

Results for average weights and weight gain showed that betaine-HCl supplementation *in ovo* significantly improved weight gain at 7days of age, with supplemented birds having higher weights ($p<0.06$). Relative organ weights at hatch day are presented in Table 3. It was observed that *in-ovo* supplementation had significant effect on all the organs except the small intestine. Yolk sac weights were not significantly influenced by supplementation. Pectoral muscle weights were observed to be highest on the un–injected control birds. This did not reflect the improvements in weights observed at hatch. It could however be that supplementation improved fluid retention in the supplemented birds. It was also observed that supplemented birds had numerically larger yolk sac compared with the un-injected birds (Figure 2), which could have resulted in the larger weights observed in the injected birds. Dos Santos *et al.* (2010) observed numerically larger relative yolk sac weights in chicks supplemented with various nutrients compared with the un-supplemented chicks. They were of the opinion that the supplementation of nutrients into the amnion helps reduce the need to use yolk content as an energy source, and may explain the higher yolk percentage in supplemented birds. Their results however showed no significant effect of *in-ovo* supplementation on liver and intestine weights.

Nguyen *et al.* (2013) observed that inclusion of betaine in diets of broiler chicks significantly improved heat production and heat increment. This could reflect in higher performance in broilers. An increase in intestinal weight and length has been associated with need to increase surface area for digestion.

Conclusion

From this study it was observed that *in-ovo* betaine supplementation improved hatchability, chick quality and hatch day weights of broiler chicks. There was also significant effect on relative organ weights and organ development of supplemented chicks.

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Table 1. Hatchability, Chick quality and Performance of *In ovo* Betaine supplemented broilers

Trial 1						
	Un-injected control	0.9% Saline	50mg betaine + 0.9% saline	100mg betaine + 0.9% saline	150mg betaine + 0.9% saline	SEM
Osmolarity* (mMol)		308.00	405.50	503.30	600.95	
Hatchability	93.75	71.88	100	59.48	48.48	
Chick weight	38.91^{a}	39.80^{a}	40.45^{ab}	40.46^{ab}	40.98^{b}	0.31
Chick length	19.46^{a}	19.10^{ab}	18.92^{bc}	18.62^{c}	18.64^{c}	0.06
Rectal temp	38.46^{a}	38.78^{a}	39.43^{b}	39.33^{b}	39.39^{b}	0.08
Trial 2						
	Un-injected control	0.9% Saline	50mg betaine + 0.9% saline	100mg betaine + 0.45% saline	150mg betaine + 0.23% saline	SEM
Osmolarity (mMol)		308.00	405.50	349.30	369.95	
Hatchability	90.30	92.65	94.20	92.65	96.55	1.41
Chick weight	39.73	40.07	40.20	40.07	40.39	0.14
Chick length	19.15^{a}	19.45^{ab}	19.38^{ab}	19.57^{b}	19.56^{b}	0.06
Rectal temp	40.00^{a}	40.12^{a}	40.56^{b}	39.65^{a}	39.95^{a}	0.04

* Osmolarity was calculated from the molecular weights of solutes.

Table 2. Average weight and weight gains of *In ovo* Betaine supplemented broilers

	Un-injected control	0.9% Saline	50mg betaine + 0.9% saline	100mg betaine + 0.45% saline	150mg betaine + 0.23% saline	SEM
Ave. Weight						
Day 0	39.73	40.07	40.20	40.07	40.39	0.14
Day 7	115.63^{a}	117.50^{a}	131.86^{b}	129.40^{ab}	118.75^{ab}	2.43

Table 3. Relative organ weights of *In ovo* Betaine supplemented broilers at Hatch day

Organs	Un-injected control	0.9% Saline	0.5% betaine + 0.9% saline	1% betaine + 0.45% saline	1.5% betaine + 0.23% saline	SEM
Liver	3.106^{ab}	3.345^{a}	2.932^{b}	2.841^{b}	2.974^{ab}	.064
Pectorals	3.611^{a}	3.050^{ab}	3.080^{ab}	2.504^{b}	2.990^{ab}	.123

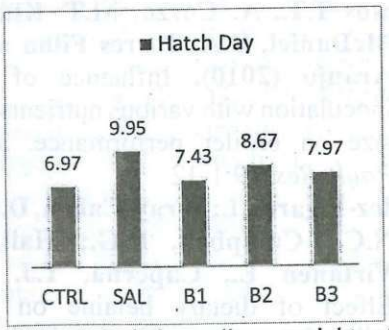


Figure 2. Relative yolk sac weights of *In ovo* betaine supplemetated broiler chicks
CTRL – control; SAL – 0.9% saline; B1 – Betaine-HCl 50mg; B2 – Betaine-HCl 100mg; B3 – Betaine-HCl 150mg