Effect of *in ovo* betaine-hydrochloride supplementation on hatchability, chick quality and organ weights of broiler chicks

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A study was carried out to assess the effect of supplementation of betaine-hydrochloride *in ovo* on hatchability, chick quality, and early post-hatch organ development of broiler chicks. A total of 425 fertile eggs of Marshall strain at 18 days of incubation were allotted to five treatments: untreated control, saline control, and betaine-hydrochloride at 5, 10 and 15 mg. Hatchability, chick quality (chick length and rectal temperature) and chick weight were assessed on hatch day. On days 0, 7 and 14 after hatching, sampled chicks were slaughtered and dissected. Visceral and humoral organs were weighed and measured to calculate relative weight change. *In ovo* betaine supplementation increased hatchability and rectal temperature in betaine-hydrochloride supplemented chicks compared with the untreated control ($P \leq 0.05$), but had no significant effect on chick length. Significant polynomial responses also observed for the absolute and relative weight of organs at days 0 and 7. Between days 0 and 14, the change in relative weight of the breast was significantly higher in chicks treated with 10 mg betaine-hydrochloride compared with those on the untreated control ($P \leq 0.05$). Feeding betaine-hydrochloride *in ovo* improved hatchability and chick quality, while its effects on the weights of organs were apparent and variable.

Keywords: Betaine, *in ovo* feeding, broilers, chick development

Chicken embryos are dependent on the nutrients within the egg, which provide the energy and building blocks required for the metabolic needs of the growing embryo during its normal 21-day incubation (Foye et al. 2006). The ability of embryos to utilise yolk nutrients is compromised by the limited availability of oxygen for aerobic metabolism until lung respiration is initiated. Therefore embryos gradually decrease fat metabolism and increase glycogen metabolism (Moran 2007). Also, yolk sac energy reserves present at the hatch may not be sufficient to supply maintenance energy requirements, and fasting effects may occur before the flock is removed from the hatchery. Weight loss between hatching and removal from the hatchery is approximately 0.18 g/h while fasting (Halevy et al. 2000).

Several alternatives have been proposed to improve performance during this initial phase such as *in ovo* feeding (Tako et al. 2004; Uni and Ferket 2004). This method makes use of the knowledge that neonatal birds naturally consume the embryonic amniotic fluid toward hatch (Romanoff 1960). Therefore, addition of a nutrient solution to the embryonic amniotic fluid delivers essential nutrients into the embryo intestine. *In ovo* injection of external nutrients has been reported to promote post-hatch growth and weight gain in birds (Tako et al. 2004).

Betaine, a methylamine with three chemically reactive methyl groups linked to a nitrogen atom, is distributed widely in animals, plants, and microorganisms. The principal physiologic role of betaine is as an osmolyte and methyl donor (transmethylation). As an osmolyte, betaine protects cells by regulating cell volume, and stabilising proteins and enzymes from environmental stress (such as low water, high salinity, or extreme temperature) (Craig 2004). In addition to dietary sources, betaine can be produced through the irreversible oxidation of choline via choline dehydrogenase and betaine aldehyde dehydrogenase. Betaine is stored at high amounts in the liver and the kidney, while concentrations in the skeletal muscles are similar to those in the plasma and brain (Slow et al. 2009). Moreover, betaine has been discussed for its role in saving methionine and choline (Fernandez-Figares et al. 2002; Rojas-Cano et al. 2011). The methionine-sparing effect of betaine makes methionine more available for protein synthesis, and the choline-sparing effect makes choline more available for lipid metabolism.

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Thus this study proposed that *in ovo* supplementation of betaine in hydrochloride form could improve the quality of hatched chicks and subsequent post-hatch organ development.

Materials and methods

A total of 425 fertile 18-day incubated eggs of comparable weight produced by a flock of 45-week-old hens from a meat-type breeder (Marshall, Obasanjo Farms, Nigeria), were used in this study. The eggs had been set in a Jamesway incubator at 37.5°C and 55% relative humidity. The eggs were examined and selected after candling. There were five *in ovo* treatments: two control treatments, untreated control and injected with 0.9% saline (saline control), and three other treatments in which saline containing dissolved quantities of 5, 10 or 15 mg of betaine were injected; 85 eggs were allocated to each of the five treatments. The wide end of the eggshell was disinfected, with 70% isopropyl alcohol whereupon an injection hole was pierced; 1mL of solution was injected into the yolk sac using a 25 mm x 21-G needle. After injection, the hole was sealed with cellophane tape and the eggs were returned to the incubators. The hatching tray was divided into individual hatching sections using thin sheets of mesh wires. Percent hatchability was calculated on the basis of the number of hatched chicks as a percentage of the *in ovo* treated eggs per replicate. At the end of incubation, all the chicks were observed and hatching weight was recorded to the nearest 0.1 g. To assess chick quality, measurements were made for chick length and rectal temperature. Also at hatch six chicks were sampled from each treatment. The weights of the chicks were determined, and the visceral and humoral organs were removed and weighed. This procedure was repeated on four sampled chicks at 7 and 14 days post-hatch.

Data were analysed using one-way ANOVA and polynomial contrasts to determine linear and quadratic responses using either of the untreated or the saline control treatments. Mean separation was carried out using Duncan's Multiple Range Test using SPSS, version 20.0. Means were considered significant at $P \leq 0.05$ and tendencial (marginal) at $P > 0.05$ to $P \leq 0.10$.

Results

Hatchability and chick quality

In ovo betaine significantly improved hatchability at 5, 10 and 15 mg, compared with the untreated control ($P \leq 0.05$), linear effect (Table 1). Saline injection, however, did not differ from the untreated control. Chick weight was similar between the treatments, although a linear increment was observed comparing the untreated control with the *in ovo* betaine levels ($P \leq 0.05$). Chick quality assessment showed that *in ovo* injection did not influence chick length, but rectal temperature was significantly higher with *in ovo* betaine at 5, 10 and 15 mg compared with the untreated control ($P \leq 0.05$; quadratic effect), with the highest temperature at 5 mg *in ovo* betaine.

Table 1: Quality of broiler chicks administered betaine *in ovo*

								UC		SC	
	UC	SC	B5 mg	B10 mg	B15 mg	SEM	P	L	Q	L	Q
Hatch (%)	86.7^{b}	92.5^{ab}	94.2^{a}	94.6^{a}	98.3^{a}	0.83	0.048	*	-	0.07	-
CW (g)	39.3	40.1	40.3	40.2	40.4	0.18	0.282	*	-	-	-
CL (cm)	19.3	19.3	19.1	19.1	19.2	0.05	0.440	-	-	-	-
RT (°C)	39.0^{b}	39.4^{ab}	39.9^{a}	39.7^{a}	39.7^{a}	0.06	0.000	***	***	-	*

^{a,b} means in a row with different superscripts are significantly different ($P \leq 0.05$)
UC = untreated control, SC = saline control, B = betaine, CW = chick weight, CL = chick length, RT = rectal temperature. L = linear, Q = quadratic at *P <≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001

Weight of organs

At hatch, absolute weight of organs was similar between the treatments for all organs except the liver which tended to reduce at 5 and 10 mg *in ovo* betaine compared with the saline control (P = 0.061). Relative weights however indicated that at 15 mg *in ovo* betaine, heart weight was significantly lower compared with the other treatments (quadratic effect using untreated control P ≤ 0.01; both linear and quadratic effects using saline control P ≤ 0.05). Breast yield (%) tended to reduce with *in ovo* feeding (P = 0.074), with a linear effect observed with untreated control (P ≤ 0.05) (Table 2).

Table 2: Hatch-day weights of organs in broiler chicks administered betaine-hydrochloride *in ovo*

								UC		SC	
	UC	SC	B5 mg	B10 mg	B15 mg	SEM	P	L	Q	L	Q
			Absolute weight (g)								
LW	41.3	40.9	39.1	41.9	41.7	0.56	0.561	-	-	-	-
Breast	1.49	1.24	1.21	1.05	1.24	0.05	0.146	0.08	-	-	-
Gizzard	2.55	2.66	2.48	2.28	2.58	0.06	0.394	-	-	-	-
Heart	0.34	0.35	0.34	0.36	0.29	0.01	0.209	-	-	0.05	-
Kidney	0.30	0.34	0.29	0.29	0.29	0.01	0.358	-	-	-	-
Liver	1.27	1.36	1.15	1.19	1.24	0.02	0.061	-	-	-	*
SI	1.59	1.69	1.53	1.55	1.81	0.06	0.597	-	-	-	-
Yolk	2.89	4.04	2.94	3.67	3.32	0.31	0.733	-	-	-	-
								-	-	-	-
			Relative weight (%)					-	-	-	-
Breast	3.61	3.05	3.08	2.50	2.99	0.11	0.074	*	-	-	-
Gizzard	6.22	6.49	6.32	5.46	6.19	0.14	0.184	-	-	-	-
Heart	0.82[ab]	0.85[ab]	0.86[a]	0.85[a]	0.71[b]	0.02	0.032	-	**	*	*
Kidney	0.74	0.82	0.74	0.71	0.69	0.02	0.304	-	-	-	-
Liver	3.11	3.35	2.93	2.84	2.97	0.06	0.107	-	-	*	*
SI	3.88	4.13	3.93	3.72	4.34	0.15	0.713	-	-	-	-
Yolk	6.97	9.96	7.43	8.67	7.97	0.75	0.745	-	-	-	-

[a,b] means in a row with different superscripts are significantly different (P ≤ 0.05), UC = untreated control, SC = saline control, B = betaine, LW = live weight, SI = small intestine.
L = linear, Q = quadratic at *P ≤ 0.05, **P ≤ 0.01

By day 7 after hatch (Table 3), the absolute weight of liver was significantly lower at 5 mg *in ovo* betaine compared with the controls (quadratic effect for both untreated and saline controls; P ≤ 0.05). Relative weights highlighted an increase in breast yield at 15 mg compared with the untreated and saline controls (linear effects P ≤ 0.05). Absolute and relative weights of organs at 14 days of age did not exhibit a significant effect of administering betaine *in ovo* to broiler chicks (Table 4).

Table 3: Day 7 weights of organs in broiler chicks administered betaine-hydrochloride *in ovo*

	UC	SC	B5 mg	B10 mg	B15 mg	SEM	P	UC L	UC Q	SC L	SC Q
			Absolute weight (g)								
LW	134	123	123	139	134	3.24	0.465	-	-	-	-
Breast	11.3	10.3	10.1	13.0	13.1	0.46	0.150	-	-	*	-
Gizzard	8.68	8.23	7.97	9.02	9.33	0.31	0.642	-	-	-	-
Heart	1.18	1.07	1.02	1.12	1.11	0.04	0.727	-	-	-	-
Kidney	1.69	1.51	1.30	1.84	1.64	0.06	0.139	-	-	-	-
Liver	5.14[a]	5.06[a]	3.86[b]	4.84[a]	5.23[a]	0.13	0.034	-	*	-	*
SI	12.5	10.3	10.0	12.3	11.0	0.30	0.057	-	-	-	-
			Relative weight (%)								
Breast	8.42	8.31	8.59	9.40	9.74	0.17	0.063	*	-	*	-
Gizzard	6.46	6.72	6.66	6.65	7.02	0.21	0.933	-	-	-	-
Heart	0.88	0.88	0.84	0.82	0.83	0.02	0.754	-	-	-	-
Kidney	1.26	1.22	1.01	1.30	1.24	0.04	0.349	-	-	-	-
Liver	3.83	4.25	3.19	3.50	4.04	0.19	0.486	-	-	-	-
SI	9.31	8.44	7.85	8.88	8.24	0.18	0.169	-	-	-	-

[a,b] means in a row with different superscripts are significantly different ($P \leq 0.05$)
UC = untreated control, SC = saline control, B = betaine, LW = live weight, SI = small intestine. L = linear, Q = quadratic at *$P \leq 0.05$

Table 4: Day 14 weights of organs in broiler chicks administered betaine-hydrochloride *in ovo*

	UC	SC	B5 mg	B10 mg	B15 mg	SEM	P
			Absolute weight (g)				
LW	258	266	277	267	257	7.53	0.915
Breast	28.3	29.6	32.2	32.6	29.7	1.29	0.806
Gizzard	15.2	15.4	13.6	15.5	14.2	0.50	0.703
Heart	1.99	2.16	2.47	2.10	2.26	0.07	0.355
Kidney	3.02	3.42	2.86	3.04	3.30	0.14	0.726
Liver	8.01	8.49	11.1	9.02	10.4	0.68	0.592
SI	17.9	18.8	18.6	16.3	17.8	0.63	0.746
			Relative weight (%)				
Breast	10.8	11.1	11.6	12.2	11.5	0.26	0.555
Gizzard	6.00	5.89	4.92	5.81	5.53	0.22	0.552
Heart	0.77	0.81	0.89	0.79	0.89	0.02	0.130
Kidney	1.17	1.29	1.03	1.15	1.30	0.05	0.399
Liver	3.15	3.19	3.96	3.41	4.02	0.23	0.610
SI	7.09	7.20	6.72	6.15	6.84	0.24	0.675

UC = untreated control, SC = saline control, B = betaine, LW = live weight, SI = small intestine.

Mean values of weights of humoral organs are presented in Table 5. Absolute and proportional weights of spleen and bursa did not differ between the treatments at hatch. However at 7 days after hatch, while weights of spleen were similar, weights of bursa tended to be higher at 15 mg *in ovo* betaine ($P = 0.076$ and $P = 0.078$, absolute and relative weights respectively), with a linear trend observed with respect to saline control ($P \leq 0.05$). This effect was however not carried on to age 14 days.

Table 5: Weight of humoral organs of chicks administered betaine *in ovo*

								UC		SC	
Day	UC	SC	B5 mg	B10 mg	B15 mg	SEM	P	L	Q	L	Q
					Bursa						
					Absolute weight (g)						
0	0.042	0.045	0.045	0.047	0.061	0.004	0.681	-	-	-	-
7	0.198	0.113	0.126	0.209	0.211	0.013	0.076	-	-	*	-
14	0.402	0.399	0.449	0.363	0.404	0.030	0.922	-	-	-	-
					Relative weight (%)						
0	0.104	0.11	0.114	0.135	0.119	0.01	0.915	-	-	-	-
7	0.148	0.089	0.103	0.153	0.157	0.009	0.078	-	-	*	-
14	0.158	0.153	0.163	0.136	0.155	0.01	0.932	-	-	-	-
					Spleen						
					Absolute weight (g)						
0	0.018	0.025	0.015	0.016	0.025	0.002	0.433	-	-	-	-
7	0.107	0.127	0.085	0.138	0.127	0.010	0.560	-	-	-	-
14	0.297	0.210	0.384	0.304	0.328	0.031	0.535	-	-	-	-
					Relative weight (%)						
0	0.044	0.060	0.037	0.036	0.059	0.005	0.446	-	-	-	-
7	0.080	0.106	0.070	0.099	0.098	0.008	0.622	-	-	-	-
14	0.116	0.078	0.137	0.112	0.126	0.011	0.510	-	-	-	-

UC = untreated control, SC = saline control, B = betaine, LW = live weight, SI = small intestine.L = linear, Q = quadratic at *$P \leq 0.05$

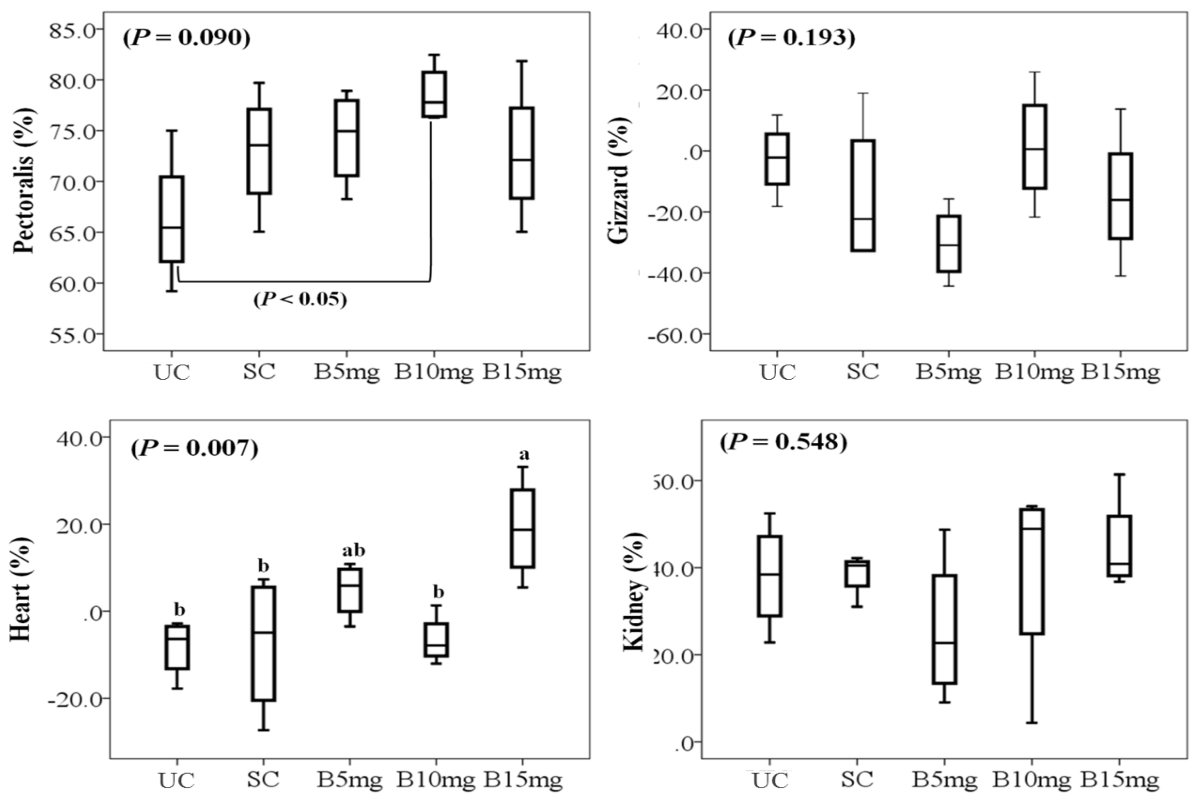

Figure 1: Box plots showing change (%) in relative weights of organs from day 0 - 14 in broiler chicks fed betaine-hydrochloride *in ovo*. Vertical boxes represent the interquartile range of means, horizontal lines within boxes indicate the median line, while upper and lower whiskers highlight the maximum and minimum observed values respectively.

UC = untreated control; SC = saline control; B = betaine.[a, b] – boxes with different superscripts are significantly different ($P \leq 0.05$)

Change in relative weight of organs

Changes in organ weights are an indication of the rate of development of organs as the birds grow. Figure 1 shows that the change in the relative weight of breast was apparently higher at 10 mg *in ovo* betaine compared with the untreated control (P = 0.090). The change in the relative weight of heart was significantly higher with *in ovo* betaine at 15mg compared with the untreated control, the saline control and 10 mg *in ovo* betaine (P = 0.007). There were no observed differences in change in relative weights of gizzard and kidney. While no differences were observed for liver, small intestine and bursa weights, change in weight of spleen tended to be lower for the saline control than for 5 and 10 mg *in ovo* betaine (P = 0.080).

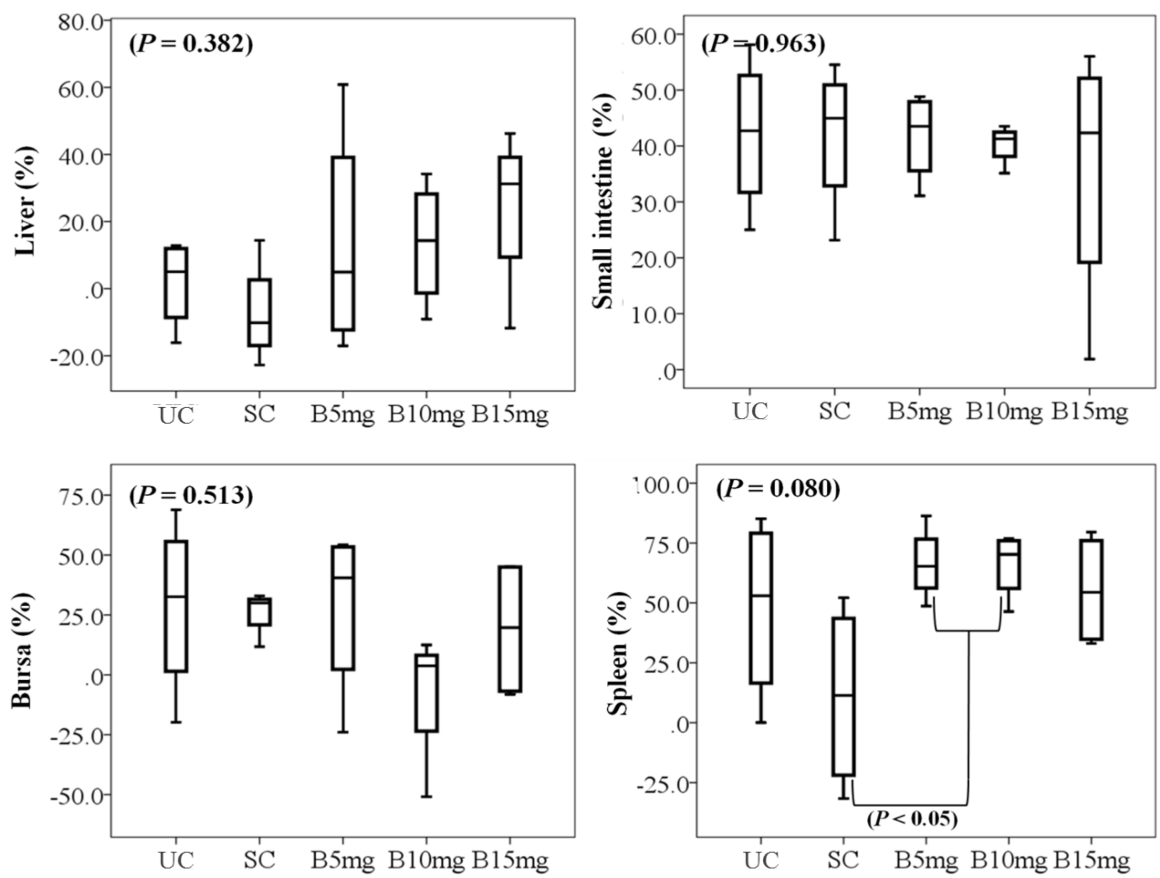

Figure 2: Box plots showing change (%) in relative weights of organs from day 0-14 in broiler chicks fed betaine-hydrochloride *in ovo*. Vertical boxes represent the interquartile range of means, horizontal lines within boxes indicate the median line, while upper and lower whiskers highlight the maximum and minimum observed values respectively.
UC = untreated control; SC = saline control; B = betaine

Discussion

Betaine has been effective as an osmolyte, by protecting cells from stress (Craig 2004), and as a methyl donor, it is required in methylation reactions for methyl donation into the gluconeogenic pathway, a major pathway at hatching. This was reflected in the improvement in hatchability of the betaine supplemented chicks. Betaine supplementation *in ovo* at very high concentrations (up to 100 mg/egg) has been observed to markedly reduce hatchability (Kadam et al. 2013), although improvement in hatch weight was also observed at this same concentration compared, with the uninjected control. Conversely, Gholami et al. (2015) administering betaine at very low concentrations of 0.25 and 0.5 mg/egg noted no significant effect on hatchability, but also observed increased absolute and relative weight of chicks at hatch, compared with the uninjected control. In addition to the aforementioned studies, hatchling weights in the current study did not differ from saline controls, indicating that besides the supplement, *in ovo* administration improves hatch weight. Previously it was reported that *in ovo* feeding ameliorates the negative effect of moisture mediated weight loss around the time of hatch (Tanimowo and Longe 2020).

Hatchling length of chick tends to be a more appropriate indicator, than weight at hatch for predicting adult-life performance (Maatjens et al. 2009), although gender must be taken into account. Studies have reported that chick length is indicative of chick development (Wolanski et al. 2006; Ipek et al. 2014), while adult-life broiler performance has also been positively correlated to chick length at hatch; a greater positive correlation was noted between broiler performance and hatchling length, than with hatch day weight, particularly when set egg was corrected for (Meijerhof 2009). It has been opined that market age body weight correlates with chick length, indicating that chick length is a more suitable predictor of the potential for growth by a chick (Wolanski et al. 2006).

With regards to rectal temperatures, studies have highlighted the influence of deviations in temperature at the embryonic stage on broiler performance in adult life (Lourens and Middlekoop 2000; Gladys et al. 2000). Differences of about 0.16°C in embryo temperature significantly influence embryo growth and feed efficiency of broilers 6 weeks post-hatch. In this present study, betaine-hydrochloride supplementation was observed to increase rectal temperature in 1 day old chicks. Addition of betaine to broiler diets has been noted to significantly impact on heat production and heat increment; Nguyen et al. (2014) observed that heat increment had no lowering effect on the nutritive efficiency of feed and therefore surmised that the higher heat production was reflective of better growth performance or alterations in the nutrient deposition ratio of broilers.

The variable effect of *in ovo* feeding on organ development in broiler chicks was also observed in this study. It was noted that feeding betaine *in ovo* had minimal effect on weight of organs in the early post-hatch developing chick. In another study (Dos Santos et al. 2010), numerically larger relative yolk sac weights were observed in chicks supplemented with various nutrients, compared with the unsupplemented chicks. Supplementation of nutrients into the amnion helps reduce the need to use yolk content as an energy source; they also did not observe any significant improvement in pectoral muscle weight when inoculating eggs with various nutrients. Hamadani et al. (2013) however stated that at the time of hatch, the small intestine of the *in ovo* supplemented bird is at a functional stage similar to that in conventionally fed 2 day old chicks. Several experiments with offspring, from young maternal flocks have demonstrated that *in ovo* feeding increases hatchling weights by 5% over controls (Uni et al. 2005) and elevates relative breast-muscle size (calculated as % of body weight) by up to 6% (Kornasio et al. 2011).

The results obtained for change in relative weights of organs highlight the rapid development of the organs during the first week of life post-hatch, by the second week change in relative weight slowed down significantly. Sparse information exists on the effect of dietary *in ovo* administration of betaine on organ development in broiler chicks. Several studies have observed various effects on breast yield and fat deposition in various avian species (Wang et al. 2004; Zhan et al. 2006; Su et al. 2009; Alirezaei et al. 2012). The osmoprotectant action of betaine influences changes in acid-base balance

(Honarbakhsh et al. 2007) and hematological indicators (Park and Kim 2017) under stress conditions.

Nutrient administration *in ovo* has been well reported to enable late-term embryos surmount the challenges of limited nutrient supply in the egg (Tako et al. 2005; Foye et al. 2006; Bohorquez et al. 2007). This occurs via improvement in enteric and critical tissue development and energy status (Uni et al. 2005; Bohorquez et al. 2008).

Conclusion

Administering betaine-hydrochloride *in ovo* improved hatchability and quality of broiler chicks. There was also apparent influence of *in ovo* feeding on weight of organs at 0 and 7 days post-hatch. Knowledge of prenatal nutrient influences will afford earlier nutritional manipulations to improve quality of chicks post-hatch performance and health.

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