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In ovo and dietary feeding of betaine to broiler chickens under heat stress conditions: Effects on hatchability, performance, body temperature and blood parameters

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Abstract The effects of *in ovo* feeding (0 and 1g/L) and dietary feeding (0 and 1g/kg diet) of betaine on hatchability, performance, body temperature and blood parameters of broiler chicks under heat stress condition, were investigated using 600 fertile eggs (Ross 308) for *in ovo* injection at 17.5d of incubation. After hatching, 192 male chickens were divided into four groups: 1- *In ovo* feeding of non-betaine solution and post-hatch diet without betaine, 2- *In ovo* feeding of non-betaine solution and post-hatch dietary feeding 1g per kg of betaine, 3- *In ovo* feeding of 1g per L betaine solution and post-hatch diet without betaine, and 4- *In ovo* feeding of 1g per L betaine solution and post-hatch dietary feeding 1g per kg of betaine. The chicks were exposed to heat stress from 7-28d for 4h/d. The results showed that body weight of hatched chicks and the hatched chick body weight to initial egg weight ratio, was significantly increased by *in ovo* feeding of betaine (P<0.05). The effects of *in ovo* feeding and dietary feeding of betaine resulted in higher feed intake (P<0.05) and daily weight gain (P<0.01) and improved feed conversion ratio (P<0.05) for 7-21d. Birds that received dietary betaine had more carcass, breast and leg weight than chickens receiving betaine-free diet at 28d (P<0.05). The experimental treatments had no significant effect on carcass yield at d 42. During heat stress between 14 and 21d age, the group that did not receive betaine (*in ovo* or dietary) had the highest cloacal temperature (P<0.01). The concentration of high-density lipoproteins, triglycerides and cholesterol in the blood of chickens that received betaine-free diet was significantly higher than other groups (P<0.05). In general, dietary betaine feeding improved the performance and carcass parameters and decreased blood lipids and cloacal temperature of chicks under heat stress conditions. However, these effects were not observed from days 28 to 42, which were free of heat stress.

Keywords: carcass, cholesterol, cloacal temperature, *in ovo* injection

Introduction

Advances in genetic selection of meat type birds have led to rapid growth with high metabolic rates, associated with more heat production (Tallentire et al., 2016). The ability of birds to regulate their body temperature is very limited; therefore, excess heat production cannot be dissipated (Yahav, 2009). Heat stress is one of the main stressors for poultry (Lara and Rostigno, 2013), which leads to increased mortality by reducing feed intake (FI) and digestibility of nutrients (Naseem et al., 2005). There are a number of nutritional strategies such as reducing protein or energy in the diet (Ratrianto et al., 2017) and using some feed supplements (Chand et al., 2016) that can be used to improve the birds' tolerance to heat stress.

Betaine (trimethyl glycine) is said to be effective in maintaining the growth and safety of chickens in heat stress conditions (Al-Sultan et al., 2019). Trimethyl glycine is a neutral chemical compound with three methyl groups serving as a methyl donor, such as choline and methionine (Ratriyanto and Mosenthin, 2018). These methyl groups are used for various metabolic processes, such as protein synthesis, energy metabolism, and conversion of homocysteine to methionine, which can be considered as an essential amino acid (Ratriyanto et al., 2009). In addition to improving the weight gain (WG) and muscle function in chicks (Chen et al., 2018), betaine also plays a vital role in the fat metabolism (He et al., 2015). The function of betaine in metabolism of lipid offers an interesting prospect in meat production to meet consumer demands for lean meat, thus betaine is often referred to as a "carcass modifier". Betaine, as a methyl donor, provides the synthesis of methyl lecithin, which can facilitate the transport of fat through the body. Also, the methyl group can be used in transmethylation reactions to synthesize carnitine and creatine, thus affecting fat metabolism in animals (Fernandez-Fígares et al., 2008). *In ovo* injection technology, in which certain substances are injected into the bird's egg or embryo during incubation, offers a new alternative to deliver bioactive compounds to the chick embryo before hatching. An alternative to early embryo feeding has been explored to improve embryonic development and chick performance. Furthermore, amnion nutrient supplementation can accelerate the birds' intestinal development and improve their ability to digest nutrients (Uni and Ferket, 2004). Recent studies showed positive effects of *in ovo* feeding on body weight (BW) gain, improvement of feed conversion ratio (FCR), and pectoral meat yield (El-Fakhrany et al., 2021; El-Kholy et al., 2019). It seems that incubation methods may be changed by this technology. Adding feed supplements to eggs with nutritious bioactive compounds by the *in ovo* technique is a new method to improve the health of the embryo and accelerate development (Shehata et al., 2021). Few reports are available on the effect of *in ovo* feeding of betaine on hatchability, performance and carcass traits, and immunity of broiler chicks (Kadam et al., 2013; Gholami et al., 2015; Maddahian et al, 2021). Therefore, the aim of the present study was to investigate the effects of *in ovo* feeding and dietary feeding of betaine on hatchability, growth and carcass performance, body temperature and blood parameters of broiler chicks under heat stress conditions.

Materials and methods

In ovo injection procedure

This experiment was conducted in the research station of Rezvan Faculty of Agriculture, Kerman, Iran. The *in ovo* solution was prepared by dissolving 1g of betaine (Betafin S1, 96% purity, Sigma Aldrich Co.) in 1L distilled water. A total of 600 fertile eggs were collected from a broiler breeder strain (Ross-308) at 43 weeks of age and used for *in ovo* feeding of betaine. At 17.5d of incubation, the eggs were candled and those which were unfertilized were discarded. The fertile eggs were weighed and divided into two groups (average weight of 55 ± 1g). Before betaine injection, the eggs were washed and disinfected using iodine tincture, and then 1mL of the betaine solution was injected into the amniotic fluid. Distilled water was injected into the betaine-free group. The *in ovo* injection was performed as recommended by Tako et al. (2004). The side of injection was sealed with melted paraffin and the eggs were returned to the hatcher. At the end of hatching, the number of hatched chickens was recorded and the chick BW was measured. Also, the hatchability and ratio of the chick BW to initial egg weight were calculated.

Birds and experimental diets

Among the hatched chickens, 192 males were selected randomly and reared according to Ross 308 broilers instructions (Aviagen, Newbridge, Scotland,UK). The chicks were weighed and divided into four groups of four replicates (cages) each (12 birds per cage) in a 2 × 2 factorial design. The cages were covered with wood shavings and the birds had free access to the diet and water. The starter and finisher diets were formulated, based on corn-soybean meal, according to the NRC (1994) recommendations (Table 1).

The experimental treatments were as follows:

1) *In ovo* feeding of free-betaine solution with free-betaine dietary feeding,
2) *In ovo* feeding of free-betaine solution with dietary feeding 1g/kg of betaine,
3) *In ovo* feeding of 1g/L betaine with free-betaine dietary feeding, and
4) *In ovo* feeding of 1g/L betaine with dietary feeding 1g/kg of betaine

The chicks were exposed daily to a high temperature condition between 12:00 and 16:00 on days 7 to 28, adjusted to the range of 36-37°C at the end of the first week and gradually decreasing to 26-27 °C in the fourth week (optimal temperature + 4 ºC). Exposure to high ambient temperature was terminated on day 28, but the experimental diets were fed until the end of the experiment on day 42. The relative humidity was maintained in the range of 65-70% throughout the breeding period. The lighting program consisted of 23h light and 1h darkness during the study. The birds were reared according to the instructions of the Iranian Council of Animal Care (1995).

Sample collection

All chicks were weighed at the beginning and at the end of each rearing period (7-21 and 21-42d). Feed intake (FI) was calculated as the difference between the amouunt of feed offered and the feed residue at the end each period. Feed conversion ratio (FCR) was calculated by dividing FI by weight gain (WG) and corrected for mortality. The health status was monitored and mortality recorded on a daily basis. At the end of weeks 2, 3 and 4, the cloacal temperature of two birds per pen was recorded by a digital thermometer before and during the high temperature conditions (at 10:00 and 14:00 h, respectively). At the age of 28 and 42d, two birds per pen were slaughtered. The carcass was then scalded, plucked, and eviscerated. The relative weight of carcass, breast, legs, wings, backbone, abdominal fat, liver and heart were measured (% of BW). Before slaughtering, a blood sample was taken from the neck artery, kept at room temperature for 3 hours, and then centrifuged (at 1500 g for 10 minutes) to obtain serum. Fasting blood sugar (FBS) and creatinine concentration were determined (laboratory kits No. 90003 and 89009 of Pars Azmoon Co., Iran, respectively) using the AUTOLAB auto-analyzer set (model PM4000, AMS Co. Romania). Total cholesterol, triglycerides (TG), and high-density lipoproteins (HDL) were measured using enzymatic colorimetric test; the adsorption at 500 nm was recorded by spectrophotometer using a mono-reagent kit (kits: CHOD, GPO-PAP and HDL-kit, Pars Azmoon Co., Iran).

Statistical analysis

This experiment was designed as a completely randomized 2×2 factorial arrangement ($Y_{ij} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$) except for the hatchability parameters that were analyzed as a completely randomized design ($Y_{ij} = \mu + T_i + e_{ij}$). The data were analyzed by using Proc GLM (SAS, 2005), and the mean differences at P<0.05 were tested using the Tukey’s test.

Table 1. Ingredients and chemical composition of the basal diets

Ingredient (% DM)	Starter diet (1-21d)	Finisher diet (21-42d)
Corn grain	54.7	62.3
Fish meal	3.00	2.00
Soybean meal	35.5	29.7
Soybean oil	3.50	3.00
Oyster shell-flour	1.20	1.25
Dicalcium phosphate	1.12	0.90
Sodium chloride	0.34	0.30
DL-Methionine	0.14	0.07
Vitamin and mineral permix[1]	0.50	0.50
Chemical composition (%)		
Metabolizable energy (MCal/kg)	3.02	3.08
Crude protein	21.7	19.3
Calcium	0.94	0.87
Available phosphorus	0.42	0.34
Digestible methionine	0.59	0.58
Digestible methionine+cystein	0.91	0.95
Dietary cation-anion balance (mEq/kg)	268	237

[1] Contained (per kg of premix): 0.2mg biotin, 12.8 mg calcium pantothenate, 60g cholecalciferol, 0.017mg cyanocobalamin, 5.2mg folic acid, 4mg menadione, 35mg niacin, 10mg pyridoxine, 33mg trans-retinol, 12 mg riboflavin, 3.0mg thiamine, 60mg dl-tocopheryl acetate, 638mg choline chloride, 3mg Cu, 25mg Fe, 1mg I, 125mg Mn, 0.3mg Co, 0.5mg Mo, 200mg Se, 60mg Zn.

Results

The *in ovo* feeding of betaine had no effect on hatchability, but the BW of hatched chicks and hatched chick BW to initial egg weight ratio increased significantly (Table 2; P<0.05).

Table 2. The effect of *in ovo* feeding of betaine on hatchability, BW of hatched chicks and chick BW to egg weight ratio (n=40)

Item	Hatchability (%)	BW of hatched chicks(g)	Hatched chick BW/ Initial egg weight (%)
IOF of non-betaine solution	85.4	55.8^{b}	75.3^{b}
IOF of 1g/L betaine solution	83.7	57.2^{a}	76.2^{a}
SEM	1.46	0.23	0.34
Sig	NS	*	*

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05; Tukey’s test)
NS= non-significant
BW= Body weight
IOF: *in ovo* feeding

As shown in Table 3, *in ovo* feeding of betaine led to an increase in FI during the 7-21d of the experiment (P<0.01), but no significant effect on FI at 21-42d and daily weight gain (DWG) and FCR at 7-42d and final BW of chicks was observed. On the other hand, dietary feeding of betaine led to an increase in FI during the 7-21d (P<0.01) and 7-42d (P<0.05) trial periods.

Table 3. The effect of *in ovo* and dietary feeding of betaine on performance of broilers chickens exposed to heat stress between days 0 and 28.

Item	Feed intake			Daily weight gain			Feed conversion ratio			Final BW	Mortality rate
	7-21d	21-42d	7-42d	7-21d	21-42d	7-42d	7-21d	21-42d	7-42d	42d	7-42d
In ovo feeding											
IOF nB	63.79^{b}	121.46	90.81	38.84	53.20	46.88	1.65	2.29	1.94	1931.5	13.55
IOF B	65.32^{a}	121.00	91.05	40.54	53.28	47.41	1.61	2.27	1.92	1953.2	12.42
SEM	0.293	0.315	0.234	0.342	0.297	0.237	0.12	0.013	0.010	9.786	1.23
Sig	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Dietary feeding											
DF nB	63.64^{b}	121.76^{a}	90.44^{b}	38.79	52.76^{b}	46.65^{b}	1.64	2.31^{a}	1.94	1921.9^{b}	13.69^{a}
DF B	65.48^{a}	120.69^{b}	91.41^{a}	40.59	53.72^{a}	47.64^{a}	1.62	2.25^{b}	1.92	1962.8^{a}	11.20^{b}
SEM	0.293	0.315	0.234	0.342	0.297	0.237	0.120	0.013	0.010	9.786	1.020
Sig	**	*	*	NS	*	*	NS	**	NS	*	*
Interaction											
IOF nB×DF nB	62.38^{b}	122.29	90.29	37.12^{b}	52.87	46.45	1.68^{a}	2.32	1.94	1913.9	13.62
IOF nB×DF B	65.21^{a}	120.63	91.33	40.55^{a}	53.53	47.31	1.61^{b}	2.26	1.93	1949.1	12.38
IOF B×DF nB	64.89^{a}	121.24	90.59	40.46^{a}	52.65	46.85	1.60^{b}	2.31	1.93	1930.1	13.58
IOF B×DF B	65.76^{a}	120.76	91.50	40.63^{a}	53.92	47.97	1.62^{b}	2.24	1.91	1976.4	12.07
SEM	0.415	0.445	0.330	0.483	0.419	0.336	0.016	0.019	0.013	13.839	1.890
Sig	*	NS	NS	**	NS	NS	*	NS	NS	NS	NS

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05; Tukey's test)
NS= non-significant, BW= Body weight
IOF nB: *In ovo* feeding of non-betaine solution, IOF B: *In ovo* feeding of 1g per L betaine solution, DF nB: dietary feeding non-betaine and DF B: dietary feeding 1g per kg of betaine.

The dietary feeding of betaine improved the DWG of chickens at 21-42 and 7-42d trial periods (P<0.05). Also, dietary betaine feeding led to improvement in FCR at 21-42d trial periods (P<0.01), but had no significant effect on FCR during the 7-21 and 7-42d. The final BW and mortality rate of chickens were increased by dietary betaine (P<0.05). The interaction effects of *in ovo* feeding and dietary feeding of betaine resulted in increasing FI (P<0.05) and DWG (P<0.01) and decreasing FCR (P<0.05) during the 7-21d period. However, the interaction effects of the experimental treatments on the performance parameters at 21-42 and 7-42d, the final BW and mortality rate of the chickens were not significant.

Table 4 shows the effects of *in ovo* feeding and dietary feeding of betaine on the relative weight of the carcass and visceral segments of broiler chickens at the age of 28d. Betaine injection increased the legs weight at 28d (P<0.05), without a significant effect on other carcass components. The dietary betaine feeding led to an increase in carcass, breast and leg weight (P<0.01) and a decrease in the liver weight (P<0.05) of chickens under heat stress.

Table 4. The effect of *in ovo* and dietary feeding of betaine on relative weight of carcass and internal organs of broiler chickens at 28d of age exposed to heat stress between days 0 and 28 (% of BW)

Item	Carcass	Breast	Legs	Wings	Neck and backbone	Abdominal fat	Liver	Heart
In ovo feeding								
IOF nB	61.99	23.75	23.15^{b}	5.37	8.41	1.83	2.87	0.079
IOF B	63.47	24.22	23.62^{a}	5.38	8.32	1.84	2.94	0.078
SEM	0.633	0.386	0.378	0.083	0.195	0.070	0.093	0.032
Sig	NS	NS	*	NS	NS	NS	NS	NS
Dietary feeding								
DF nB	60.58^{b}	22.85^{b}	22.27^{b}	5.29	8.23	1.91	3.08^{a}	0.78
DF B	64.89^{a}	25.12^{a}	24.50^{a}	5.47	8.49	1.76	2.73^{b}	0.79
SEM	0.633	0.386	0.378	0.083	0.195	0.070	0.093	0.032
Sig	**	**	**	NS	NS	NS	*	NS
Interaction								
IOF nB×DF nB	58.75^{c}	21.88^{c}	21.32^{c}	5.31	8.29	1.88	3.12	0.79
IOF nB×DF B	65.25^{a}	25.61^{a}	24.98^{a}	5.43	8.52	1.78	2.61	0.79
IOF B×DF nB	62.42^{b}	23.82^{b}	23.22^{b}	5.26	8.17	1.94	3.04	0.77
IOF B×DF B	64.54^{ab}	24.62^{ab}	24.01^{ab}	5.50	8.46	1.74	2.85	0.78
SEM	0.895	0.546	0.535	0.118	0.275	0.099	0.132	0.045
Sig	*	*	*	NS	NS	NS	NS	NS

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05; Tukey's test)
NS= non-significant
IOF nB: *In ovo* feeding of non-betaine solution, IOF B: *In ovo* feeding of 1g per L betaine solution, DF nB: dietary feeding non-betaine and DF B: dietary feeding 1g per kg of betaine.

The interaction effects of *in ovo* feeding and dietary feeding of betaine on carcass weight, breast and legs were significant. Chickens that received dietary betaine had more carcass, breast and leg weights (P<0.05). There was no significant difference in the weight of other carcass components under the influence of the interaction effects of *in ovo* feeding and dietary feeding of betaine at 28d. As shown in Table 5, *in ovo* feeding of betaine had no significant effect on the weight of carcass components at 42d, but dietary feeding of betaine increased the relative weights of carcass, breast and legs (P<0.01) and decreased the liver weight (P<0.05) at 42d. The interaction effects of *in ovo* feeding and dietary feeding of betaine on the weight of carcass components at 42d were not significant.

Table 5. The effect of *in ovo* and dietary feeding of betaine on relative weight of carcass and viscera segments of broiler chickens at 42 d of age exposed to heat stress between days 0 and 28 (% of BW)

Item	Carcass	Breast	Legs	Wings	Neck and backbone	Abdominal fat	Liver	Heart
In ovo feeding								
IOF nB	60.78	21.38	26.22	5.42	7.25	2.08	2.81	0.65
IOF B	61.38	21.62	26.46	5.65	7.35	1.93	2.71	0.64
SEM	0.502	0.175	0.193	0.128	0.132	0.127	0.096	0.025
Sig	NS	NS	NS	NS	NS	NS	NS	NS
Dietary feeding								
DF nB	60.20^{b}	21.22^{b}	26.01^{b}	5.50	7.22	2.35^{a}	2.71	0.64
DF B	61.96^{a}	21.79^{a}	26.67^{a}	5.56	7.38	1.67^{b}	2.81	0.65
SEM	0.502	0.175	0.193	0.128	0.132	0.127	0.096	0.025
Sig	*	*	*	NS	NS	**	NS	NS
Interaction								
IOF nB×DF nB	60.17	21.17	26.01	5.33	7.19	2.46	2.85	0.65
IOF nB×DF B	61.39	21.59	26.43	5.50	7.30	1.71	2.76	0.65
IOF B×DF nB	60.23	21.27	26.00	5.66	7.25	2.24	2.56	0.63
IOF B×DF B	62.53	21.98	26.92	5.63	7.45	1.62	2.85	0.66
SEM	0.711	0.247	0.272	0.181	0.187	0.180	0.136	0.036
Sig	NS	NS	NS	NS	NS	NS	NS	NS

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05; Tukey's test)

NS= non-significant

IOF nB: *In ovo* feeding of non-betaine solution, IOF B: *In ovo* feeding of 1g per L betaine solution, DF nB: dietary feeding non-betaine and DF B: dietary feeding 1g per kg of betaine.

In ovo feeding of betaine had no effect on the cloacal temperature of chickens before and during heat stress (Table 6). Also, dietary betaine feeding had no effect on cloacal temperature before heat stress, but during heat stress, it significantly decreased the cloacal temperature at 14 and 21d (P<0.01). However, the effect of dietary feeding of betaine on cloacal temperature during stress was not significant at 42d.

Table 6. The effect of *in ovo* and dietary feeding of betaine on cloacal temperature (˚C) of broilers before and during heat stress

Item	Day 14		Day 21		Day 28	
	Before HS	During HS	Before HS	During HS	Before HS	During HS
In ovo feeding						
IOF nB	39.53	41.13	39.71	40.63	39.80	40.68
IOF B	39.58	40.81	39.86	40.40	39.54	40.48
SEM	0.186	0.112	0.152	0.134	0.096	0.110
Sig	NS	NS	NS	NS	NS	NS
Dietary feeding						
DF nB	39.74	41.37^{a}	39.56	40.85^{a}	39.63	40.64
DF B	39.35	40.56^{b}	40.00	40.18^{b}	39.71	40.52
SEM	0.186	0.112	0.152	0.134	0.096	0.110
Sig	NS	**	NS	**	NS	NS
Interaction						
IOF nB×DF nB	39.72	41.73^{a}	39.60	41.15^{a}	39.80	40.65
IOF nB×DF B	39.33	40.53^{c}	39.81	40.11^{b}	39.80	40.70
IOF B×DF nB	39.76	41.02^{b}	39.51	40.56^{b}	39.46	40.63
IOF B×DF B	39.39	40.60^{bc}	40.20	40.25^{b}	39.62	40.33
SEM	0.263	0.158	0.214	0.188	0.135	0.155
Sig	NS	**	NS	**	NS	NS

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05; Tukey's test)

NS= non-significant

HS: Heat stress

IOF nB: *In ovo* feeding of non-betaine solution, IOF B: *In ovo* feeding of 1g per L betaine solution, DF nB: dietary feeding non-betaine and DF B: dietary feeding 1g per kg of betaine.

The interaction effects of *in ovo* feeding and dietary feeding of betaine on the cloacal temperature of chickens before heat stress were not significant, but during heat stress on 14 and 21d, the group that did not receive betaine (neither *in ovo* and nor dietary) had the highest cloacal temperature (P<0.01). The interaction effects of the experimental treatment on the cloacal temperature of chickens at 42d were not significant. *In ovo* feeding of betaine had no significant effect on blood parameters of chickens at 28 and 42d. Dietary feeding of betaine did not impact on FBS of chickens at 28 and 42d (Table 7). Blood levels of HDL, TG, creatinine (P<0.01) and cholesterol (P<0.05) at 28d of age were significantly decreased by dietary betaine feeding. Also, at 42d, blood HDL and TG levels were significantly decreased by dietary feeding of betaine (P<0.05).

Table 7. The effect of *in ovo* and dietary feeding of betaine on blood parameters of broiler chickens at 28 and 42d exposed to heat stress between days 0 and 28

Item	28d					42d				
	FBS	HDL	TG	Cholesterol	Creatinine	FBS	HDL	TG	Cholesterol	Creatinine
In ovo feeding										
IOF nB	292.50	107.83	182.73	1580.23	0.055	276.50	92.27	142.05	152.53	0.646
IOF B	294.67	107.00	182.99	151.42	0.053	286.13	91.34	140.13	150.63	0.653
SEM	1.75	1.446	1.760	3.502	0.001	4.752	0.952	0.918	1.226	0.022
Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Dietary feeding										
DF nB	293.50	113.46^{a}	188.74^{a}	158.16^{a}	0.058^{a}	279.46	93.31^{a}	142.76^{a}	153.00	0.648
DF B	293.67	101.38^{b}	176.93^{b}	143.49^{b}	0.051^{b}	283.17	60.29^{b}	139.42^{b}	150.17	0.653
SEM	1.799	1.446	1.760	3.503	0.001	4.752	0.952	0.918	1.226	0.022
Sig	NS	**	**	*	**	NS	*	*	NS	NS
Interaction										
IOF nB×DF nB	293.00	116.42^{a}	192.32^{a}	159.96^{a}	0.060	274.67	94.75	144.96	154.49	0.650
IOF nB×DF B	292.00	99.25^{b}	173.14^{c}	140.49^{b}	0.050	278.33	89.78	139.14	150.58	0.643
IOF B×DF nB	294.00	110.50^{a}	185.16^{ab}	156.36^{a}	0.055	284.25	91.87	140.56	151.50	0.645
IOF B×DF B	295.33	103.50^{b}	180.83^{b}	146.49^{b}	0.053	288.00	90.81	139.70	149.76	0.663
SEM	2.544	2.046	2.489	4.953	0.002	6.721	1.346	1.298	1.734	0.032
Sig	NS	*	*	*	NS	NS	NS	NS	NS	NS

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05; Tukey's test)
NS= non-significant
FBS: Fasting blood sugar, HDL: High density lipoprotein, TG: Triglyceride
IOF nB: *In ovo* feeding of non-betaine solution, IOF B: *In ovo* feeding of 1g per L betaine solution, DF nB: dietary feeding non-betaine and DF B: dietary feeding 1g per kg of betaine.

The interaction effects of *in ovo* feeding and dietary feeding of betaine on FBS and creatinine at 28d were not significant, but blood HDL, TG and cholesterol levels in chickens that did not receive dietary betaine were significantly higher than those in other groups (P<0.05). At 42d, none of the blood parameters were significantly affected by the interaction effects of *in ovo* feeding and dietary feeding of betaine.

Discussion

This study aimed to evaluate the effects of *in ovo* feeding and dietary feeding of betaine on modulating the harmful effects of heat stress in broilers. Chickens are hatched with an underdeveloped digestive system (Geyra et al., 2001). *In ovo* feeding is considered as an applicable technique to ensure the chicks' development in the final period of incubation (Uni and Ferket, 2004). It is possible that betaine helps the absorption of substances with high osmotic pressure in the lumen and leads to increasing intestinal development (Dos Santos et al., 2019). According to the results, there was no significant difference in hatchability between betaine-free and betaine treatment by *in ovo* feeding, while the BW of hatched chicks and ratio of hatched chick BW to initial egg weight were improved. Betaine is commonly perceived as an important nutrient for embryonic and fetal development (Lever and Slow, 2010), while the effects of prenatal betaine in newborn animals are less well known (Hu et al., 2015). Gholami et al. (2015) obtained similar results by *in ovo* injection of 0.25, 0.375 and 0.5 mg of betaine. They reported that the increase in post-hatch BW shown may be due to *in ovo* injection of betaine improving body function of chicken embryos. On the other hand, it is reported that betaine acts as a methyl donor and prevents methionine that is naturally found in eggs, from degradation to harmful metabolizable products and spares methionine for growth (Zhan et al., 2006; Hu et al., 2015). Betaine also causes the conversion of homocysteine to methionine (Hu et al., 2015), which prevents the harmful effects of homocysteine accumulation (Saeed et al., 2017). Furthermore, betaine prevents dehydration and maintains water retention by maintaining the protective osmolytic activity (Maddahian et al., 2017; Saeed et al., 2017).

The decrease in FI occurring in heat stress conditions may be due to lower energy requirements for body temperature maintenance (Freeman, 1988). Consistent with our results, it is reported that, feeding betaine to chickens significantly increased FI (Awad et al., 2014; Sakomura et al., 2013). The results of the present study are in agreement with those found by Kadam et al. (2013), who reported that the effect of betaine on BW and FCR from hatch to 21d was not significant. On the other hand, in the present study, the dietary feeding of betaine led to an increase in FI, DWG and FCR at 21-42d. Similarly, Zou et al. (2002) observed an improvement in FCR in laying hens with dietary betaine feeding. The increment in BW gain may be attributed to the ability of betaine to improve the nutrient digestibility (Eklund et al., 2006a, b). Betaine leads to the compensation of osmotic disturbance during heat stress in broilers by improving the function of intestinal epithelium and increases nutrient absorption (Sakomura et al., 2013). In addition, betaine plays a role in the metabolism of protein and energy (Eklund et al., 2005). In contrast, Matthews et al. (1997) reported that betaine in the diet decreased DWG and FI in uninfected chicks, but increased DWG and FI in coccidiosis-infected chicks. It seems that the beneficial effects of betaine feeding are more apparent under stress conditions. Accordingly, our findings indicated that, the effect of betaine on FI, DWG and FCR was significant in chickens exposed to heat stress. In betaine-free chickens (neither *in ovo* nor dietary), the lowest FI and DWG and the highest FCR were observed. In general, it has been reported that betaine supplementation improves BW gain and FI in broilers under heat stress conditions (Farooqi et al., 2005, Singh et al., 2015). This may be due to the betaine's osmotic effects that cause an increment in water retention and improve energy and protein digestibility by strengthening the structure and function of the intestinal epithelium and sparing essential amino acids (Sakomura et al., 2013). In contrast, Zulkifli et al. (2004) reported that betaine feeding under heat stress conditions had no effect on DWG and FCR in broilers.

In the present study, the treatments that received dietary betaine had higher relative weights of carcass, breast and legs at 28d. This may be explained by more effective use of protein for lean accumulation, which is supported by decreased levels of blood urea nitrogen and metabolizable energy requirements and the increased nitrogen retention (Eklund et al., 2005). It is reported that, betaine can decrease the rate of protein turnover; leading to higher nitrogen retention, which in turn reduces carcass fat (Coma et al., 1995; Eklund et al., 2005). However, none of the carcass and viscera segments weight was affected by experimental treatments at 42d. Due to the presence of heat stress conditions, betaine effects are significant until the age of 28d, because betaine improves the bird's capacity to tolerate high temperatures (Ratriyanto and Mosenthin, 2018). In agreement with the results of the present study, several authors reported an increment in breast meat yield in broiler chicken (Zhan et al., 2006), turkeys (Noll et al., 2002) and meat ducks (Wang et al., 2004) with betaine feeding, while they also reported a reduction in abdominal fat. These findings are in accordance with our results at 42d, which showed a significant reduction in the abdominal fat of chickens that received dietary betaine. On the other hand, the increased percentage of carcass lean may be related to the greater accessibility of cysteine and methionine for retention of protein (McDevitt et al., 2000). Greater utilization of amino acids for synthesis of protein may lead to a decrease in amino acids available for deamination and adipose tissue synthesis (Wallis, 1999). Therefore, with betaine supplementation, changes in enzymes and hormones involved in the regulation of fat synthesis and breakdown have been observed (Huang et al., 2006; Zhan et al., 2006). Furthermore, Saunderson and McKinlay (1990) reported that betaine provides methyl groups for lecithin synthesis, which facilitates the transport of fat through the body. Nevertheless, Fernández-Fígares et al., (2008) showed that feeding betaine to broilers did not significantly improve carcass characteristics. These differences may be related to the concentration of betaine in the diet, the duration and severity of heat stress and even the composition of the diet (Ismail et al., 2017).

One of the best metabolic rate indicators in broilers is cloacal temperature, which increases when birds are exposed to heat stress. Similar to the report of Sayed and Downing (2015) and Fernandes et al. (2013), our findings indicated that, betaine was effective in reducing the bird's body temperature during heat stress. A lower rectal temperature on days 14 and 21 in birds that received betaine (*in ovo* or dietary) may be explained by a reduction in metabolic rates. Betaine, as an osmolyte, has a special role in preserving cellular water and ion balance in poultry, and improves the chick's ability to tolerant high temperatures. Betaine reduced the rectal temperature of chickens from 43.2°C to 41.9°C (Zhan et al., 2006). In contrast, Gudev et al. (2011) reported that betaine feeding had no effect on reducing the cloacal temperature of chickens under heat stress conditions. Contrary to our prediction, although heat stress conditions were still maintained at the age of 28d, the experimental treatments had no effect on the body temperature of chicks at this age. The ability of chicks to regulate body temperature after birth appears to increase with age through neural and hormonal actions (Nichelmann and Tzschentke, 2002; Tan et al., 2010).

The lipid profile in serum is a significant index of lipid metabolism in the body. At high ambient temperature, due to the decrease in FI, the required energy is supplied through fat lipolysis, which leads to an increase in serum cholesterol and TG concentrations (Rashidi et al., 2010). In our study, the dietary feeding of betaine reduced the serum concentrations of HDL, TG and cholesterol in the chicks under heat stress. In line with our findings, it was previously reported that betaine increased the lipase activity and reduced the TG and cholesterol level in blood of laying hens (Ratriyanto et al., 2009). Meanwhile, betaine increases the availability of choline and provides more choline for the synthesis of very low-density lipoprotein (VLDL). Production of VLDL prevents fat deposition in the liver and leads to an increase in fat removal from the liver (Yao and Vance, 1989). Singh et al. (2015) reported that, blood TG declined when betaine was administered at 2g per kg diet, but was ineffective at 1.3g per kg. Limited reports have been published in this regard, and most of the reports indicated limited effects of betaine on blood TG (Konca et al., 2008; Baghaei et al., 2011). It appears that the supplementation of betaine may lead to a reduction in blood TG because the hormone-sensitive lipase activity, which catabolizes TG, is decreased (Zhan et al., 2006; He et al., 2015).

Conclusions

The results of this study showed that, the effect of *in ovo* feeding of betaine was not seen in the long term because betaine is a water-soluble substance and is quickly excreted from the body through urine. Meanwhile, dietary betaine feeding improved the performance and carcass parameters and decreased blood lipids and the cloacal temperature of chicks under heat stress conditions. However, these effects were not observed from days 28 to 42, which were free of heat stress. It is suggested that betaine supplement may be used in raising broilers exposed to high temperatures.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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