



Effect of dietary betaine supplementation on egg quality, semen quality, hematology, fertility and hatchability in broiler breeders

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ABSTRACT

An experiment was conducted to establish the growth response, egg quality, semen quality, hematological attributes, fertility and hatchability in broiler breeders supplemented with graded levels of betaine. The broiler breeders (CARIBRO-VISHAL) of about 38 weeks divided into four treatments viz. T1 (basal diet), T2 (T1 + 1 g), T3 (T1 + 2 g) and T4 (T1 + 3 g betaine/kg) consisting of 24 female birds and 6 male birds per treatment with three replicates of 8 female and 2 male birds each in completely randomized design. Hen day egg production (HDEP) was significantly increased in betaine supplemented groups. Semen concentration was found to be significantly improved following betaine supplementation with highest increment in T4 group. Mass motility in T3 group during 4th week of experimental trial was found to be significantly increased. In female birds, a significant difference in RBC count, PCV, MCH and MCHC was observed among the treatments during 2nd week of trial. Fertility and hatchability (TES: total eggs set; FES: fertile eggs set) was increased in betaine supplemented groups with highest increment in T4 treatment group. Total embryonic mortality was lowest for T4 treatment group. Thus, it can be concluded that dietary supplementation of betaine at 3 g/kg was helpful in improving the reproductive performance in broiler breeders.

Keywords: Betaine, Broiler breeders, Fertility, Hatchability, Semen quality

Fertility and hatchability are the two attributes that highly influence the supply of chicks. A balanced nutritional status of parent stock is crucial for the transfer of nutrients to eggs which is a prerequisite for the normal development of the embryos (Cadogan *et al.* 2014). Higher metabolic demand due to fast growing embryos, an insufficient nutrient reserve may be manifested as poor hatchlings, retarded body weight and muscle gain (Uni *et al.* 2004). Stress, a major problem in broiler breeder production occurs as a result of confinement, bigger body size and environmental factors. In tropics, high temperature with humidity is a contributing factor for stress. Heat stress not only negatively affects production performance but imparts welfare issues, such as increased mortality, discomfort and dermatitis (Nawab *et al.* 2018). To tackle these major challenges there is a need to develop some exogenous strategies which can supply the balance nutrients and improving stress tolerance.

Betaine would provide a strategic solution for these challenges. A trimethyl derivative of glycine, betaine is a metabolite of plant and animal tissues and functions by: (1) protecting cells against osmotic inactivation which helps in maintaining cell volume under heat stress condition;

(Saeed *et al.* 2017) and (2) methyl group donor via trans methylation pathway to synthesize critical metabolites and regulate gene expression through DNA methylation (Anderson *et al.* 2012). Its epigenetic role helps in the synthesis of biomolecules (DNA, RNA and protein as well as choline) and improves immune functions. Betaine, with its dual function, has been evaluated for its various effects including nutrient sparing effect, improving performance efficiency, altering the body fat (Rao *et al.* 2011) and reducing mortality (Esteve-Garcia and Mack 2000, Lukic *et al.* 2012) in heat stress birds. Cadogan *et al.* (2014) stated that high level of blood homocysteine reduces chick survivability. Betaine supplementation reduce the homocysteine concentration and increases the embryonic survivability and in turn hatchability (Van Wettere *et al.* 2012). So, based on all above background, we have designed present study to evaluate the effect of betaine supplementation in broiler breeders during summer season.

MATERIALS AND METHODS

The experiment was conducted at during June to July month of year and was approved by the Institute Animal Ethics Committee (IAEC). Betaine as Betaine hydrochloride (feed grade 96%) was purchased from Indian Trading Bureau Private Limited, Kolkata, West Bengal, India.

Experimental birds and design: Ninety six female and

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24 male broiler breeders (CARIBRO-VISHAL) of about 38 weeks age were selected for the study. The birds were divided into four treatments, viz. T1 (control), T2, T3 and T4 (24 female birds per treatment and 6 male birds per treatment) with three replicates of 8 female and 2 male birds each. Male and female birds were reared separately with respective breeder ration. All the birds were housed in battery cages with uniform and standard management condition with 16h lighting. The mean maximum and minimum temperature was 39° and 21°C, respectively. The relative humidity during the study period was between 45–50%. Experimental diets contained 0, 1, 2 and 3 g betaine/kg of diet in T1, T2, T3 and T4, respectively. The basal diet was formulated for female (16% CP, 2800 kcal/kg ME, 3.5% Ca, 0.44% Avil P) and male (15% CP, 2750 kcal/kg ME, 1% Ca, 0.44% Avil P) as per guidelines of ICAR 2013.

Determination of body weight and egg quality: Data regarding fortnight body weight (BW) (g) change in the control and experimental groups were recorded during entire feeding trial on individual basis. Forty eggs (ten eggs per replicate) were collected on 2nd, 4th and 6th week of feeding trial to assess the quality of egg. External egg quality viz. egg weight, shape index, shell color, shell thickness and internal egg qualities, albumen index, haugh unit, yolk index and yolk color were evaluated.

Determination of semen quality: Twenty four semen samples from the male broiler breeders (six male per treatment) were collected on 2nd, 4th and 6th week of feeding trial before feeding and watering by the abdominal massage method (Burrows and Quinn 1937) which examined for concentration and mass motility.

Determination of hematological parameters: Blood samples were collected (1 ml) from the jugular vein using 24 gauge needle in K3-EDTA tubes on 2nd, 4th and 6th week (8 female birds per treatment and 6 male birds per treatment). This was analyzed for RBC, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) by autoanalyser (Abacus Junior Vet5, Diatron, USA).

Determination of fertility and hatchability: At the end of the experimental period (42nd days), the fertility and hatchability performance were assessed. Semen samples were pooled from their respective treatment groups and artificial insemination (Burrows and Quinn 1937) was done in their respective dietary treatment female birds. The eggs were collected up to 7 days post-AI and transferred to the hatchery for the evaluation of fertility, hatchability and embryonic mortality.

Statistical analysis: Data generated from different treatments were analyzed using completely randomized design (CRD) for their statistical significance by Snedecor and Cochran (1989) method. All data were statistically analyzed using SPSS software package version 20.0. Variables having different observations were analyzed following Duncan's multiple range test (Duncan, 1965).

Table 1. Effect of betaine supplementation on egg production and quality

Treatment	2 nd week	4 th week	6 th week
<i>Hen day egg production (%)</i>			
T1	41.14 ^a ±1.70	49.51 ^a ±4.24	51.80 ^a ±1.24
T2	49.81 ^b ±1.69	51.44 ^{ab} ±1.16	56.42 ^b ±2.40
T3	58.78 ^c ±1.31	58.42 ^{ab} ±1.33	56.80 ^b ±2.35
T4	55.89 ^{bc} ±1.63	61.00 ^b ±1.97	57.29 ^c ±1.17
P-value	0.000	0.010	0.001
<i>Egg weight (g)</i>			
T1	69.74±0.99	68.25±3.81	64.02 ^a ±1.79
T2	63.17±5.86	71.02±0.55	69.84 ^b ±1.34
T3	70.51±2.97	73.58±2.00	77.34 ^c ±0.94
T4	67.42±2.05	71.05±2.55	72.00 ^{bc} ±2.74
P-value	0.470	0.543	0.020
<i>Shape index (%)</i>			
T1	72.08±1.12	75.66±0.94	71.99±2.72
T2	73.71±1.51	77.11±0.77	75.10±2.00
T3	77.54±3.26	76.56±1.45	70.28±2.03
T4	74.53±0.64	73.48±1.23	74.71±1.86
P-value	0.281	0.165	0.384
<i>Albumen index</i>			
T1	81.99±3.28	88.26±5.04	86.83±2.94
T2	85.63±2.29	83.54±2.62	85.19±2.49
T3	88.23±3.73	86.50±2.53	85.13±4.10
T4	87.70±3.34	86.89±3.75	95.38±1.18
P-value	0.526	0.825	0.790
<i>Albumen height (mm)</i>			
T1	8.93 ^{ab} ±0.24	8.08±0.40	8.58±0.49
T2	8.78 ^a ±0.53	8.93±0.45	8.40±0.32
T3	10.13 ^b ±0.49	7.93±0.26	9.50±0.82
T4	8.70 ^a ±0.33	8.43±0.85	9.63±0.24
P-value	0.008	0.584	0.273
<i>Yolk weight (g)</i>			
T1	44.07±1.05	43.75±0.80	42.80±1.46
T2	43.05±1.45	44.63±0.19	44.50±0.29
T3	45.88±1.11	44.15±0.75	46.05±1.44
T4	45.35±0.81	45.98±0.66	44.98±0.79
P-value	0.328	0.136	0.267
<i>Yolk colour</i>			
T1	8.25±0.25	4.75±0.48	5.75±0.85
T2	8.50±0.65	5.50±1.19	6.25±1.31
T3	8.25±0.63	4.25±0.25	7.50±1.44
T4	8.25±0.48	5.25±0.48	7.25±0.75
P-value	0.981	0.607	0.667
<i>Shell colour</i>			
T1	7.25±0.63	5.00±0.41	4.75±0.48
T2	7.25±1.38	6.50±0.65	6.00±0.82
T3	6.00±0.41	7.25±0.48	7.00±0.71
T4	8.00±1.08	6.25±0.48	7.25±1.31
P-value	0.539	0.550	0.230

T1, control group; T2, control+1 g betaine/kg diet; T3, control+2 g betaine/kg diet; T4, control+3 g betaine/kg diet.

^{a-c}Means±SE within a column with no common superscripts differ significantly (P<0.05).

RESULTS AND DISCUSSION

Effect on body weight: Effect of dietary betaine supplementation on fortnight body weight (g) during the feeding trial is summarized in Fig. 1. There was no significant ($P>0.05$) effect on body weight (g), in both the sexes of broiler breeders following betaine supplementation. Betaine supplementation did not affect the body weight of broiler breeders. The present result was in line with Shakeri *et al.* (2018) who did not observe any significant effect on body weight of broilers following betaine supplementation. There is no work available on study of betaine supplementation on broiler breeder's body weight. The non-significant change is may be due to less dose or higher body weight of breeder compare to broilers.

Effect on egg quality: Hen day egg production (HDEP) was significantly ($P<0.01$) increased following dietary supplementation of betaine. It was highest in T3 treatment group during 2nd week of feeding trial, whereas, during 4th and 6th week of trail it was highest for T4 treatment group as compared to others (Table 1). Egg weights were comparable ($P>0.05$) among the treatments during 2nd and 4th week, whereas, it was highest ($P<0.05$) in T3 treatment

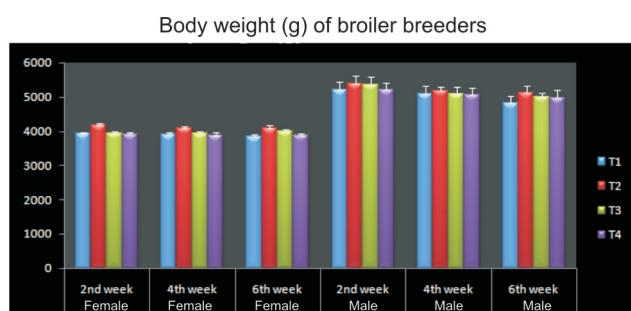


Fig. 1. Effect of betaine supplementation on fortnightly body weight (g) of broiler breeders. T1, control group; T2, control +1 g betaine/kg diet; T3, control + 2 g betaine/kg diet; T4, control + 3 g betaine/kg diet.

Table 2. Effect of betaine supplementation on semen quality

Treatment	2 nd week	4 th week	6 th week
<i>Concentration</i> ($\times 10^9$ cells/ml)			
T1	1.47 ^a $\pm$ 0.13	1.56 ^a $\pm$ 0.15	1.56 ^a $\pm$ 0.15
T2	1.87 ^{ab} $\pm$ 0.22	1.81 ^{ab} $\pm$ 0.13	1.98 ^b $\pm$ 0.02
T3	1.93 ^b $\pm$ 0.10	2.17 ^{bc} $\pm$ 0.12	2.17 ^{bc} $\pm$ 0.12
T4	2.46 ^c $\pm$ 0.09	2.54 ^c $\pm$ 0.21	2.36 ^c $\pm$ 0.11
P value	0.001	0.001	0.000
<i>Mass motility</i> (%)			
T1	65.00 $\pm$ 2.89	73.33 ^a $\pm$ 3.33	80.67 $\pm$ 4.67
T2	68.33 $\pm$ 8.33	82.00 ^{ab} $\pm$ 4.36	82.33 $\pm$ 3.18
T3	76.67 $\pm$ 3.33	95.67 ^b $\pm$ 1.20	91.00 $\pm$ 4.16
T4	66.67 $\pm$ 14.53	87.67 ^{ab} $\pm$ 6.36	91.33 $\pm$ 1.76
P value	0.785	0.032	0.142

T1, control group; T2, control+1 g betaine/kg diet; T3, control+2 g betaine/kg diet; T4, control+3 g betaine/kg diet.

^{a-c}Means $\pm$ SE within a column with no common superscripts differ significantly ($P<0.05$).

Table 3. Effect of betaine supplementation on blood hematology in broiler breeders

Parameter	Female				Male				P value
	T1	T2	T3	T4	T1	T2	T3	T4	
2 nd week									
RRBC (×10 ⁶ /μl)	1.76 ^a ±0.10	2.16 ^b ±0.10	1.95 ^{ab} ±0.06	1.78 ^a ±0.08	2.54±0.10	2.43±0.15	2.38±0.24	2.47±0.14	0.917
Hb (g%)	12.13±0.65	12.18±0.46	12.05±0.48	12.00±0.71	17.40±0.72	16.13±0.39	15.27±1.22	16.17±0.87	0.419
PCV (%)	38.73 ^a ±0.83	42.58 ^b ±1.06	40.95 ^{ab} ±0.78	39.43 ^a ±0.88	28.75±1.40	27.16±1.71	26.29±2.06	27.28±2.12	0.820
4 th week									
RRBC (×10 ⁶ /μl)	1.85±0.14	1.93±0.06	1.92±0.06	1.86±0.05	2.45±0.03	2.56±0.10	2.75±0.08	2.59±0.16	0.308
Hb (g%)	11.93±0.33	12.23±0.27	12.43±0.27	12.30±0.27	17.53±0.03	19.07±0.62	19.53±0.47	18.73±0.75	0.135
PCV (%)	40.18±0.96	40.72±0.66	41.09±0.80	40.02±0.47	28.01±0.18	29.59±1.21	31.38±0.62	29.34±1.57	0.231
6 th week									
RRBC (×10 ⁶ /μl)	1.57±0.30	1.57±0.05	1.44±0.08	1.61±0.03	2.2±0.13	2.67±0.19	2.24±0.41	2.47±0.14	0.523
Hb (g%)	13.85±1.55	12.48±0.28	12.40±0.46	14.10±0.07	19.83±2.88	19.9±1.20	18.6±1.50	18.63±0.84	0.917
PCV (%)	40.72±3.40	38.26±0.59	36.82±1.13	38.90±0.42	25.71±1.85	30.56±2.07	26.09±4.81	28.54±1.57	0.622
T1, control group; T2, control+1 g betaine/kg diet; T3, control+2 g betaine/kg diet; T4, control+3 g betaine/kg diet. RBC, Red Blood Corpuscles; Hb, Hemoglobin; PCV, Packed Cell Volume.									

T1, control group; T2, control+1 g betaine/kg diet; T3, control+2 g betaine/kg diet; T4, control+3 g betaine/kg diet. RBC, Red Blood Corpuscles; Hb, Hemoglobin; PCV, Packed Cell Volume.

group as compared to T1 and T2 groups at 6th week of trial. While the T4 group showed an intermediate response. Other parameters such as shape index, albumen index, albumen height (mm), yolk weight (g), yolk and shell colour remained non-significant ($P>0.05$) among the treatments.

In present study, Hen day egg production (HDEP) was increased significantly with betaine supplementation, however, egg quality traits were unaffected. The study regarding betaine supplementation and its effect on fertile egg production are very scanty. Gudev *et al.* 2011 reported that betaine supplementation improved egg production without affecting the quality of egg laid. Hasan and Abass 2013 also reported an increased HDEP with betaine supplemented groups compared to control in laying hens. Heberle *et al.* 2016 reported that weekly egg production was increased with betaine whereas, egg quality traits such as; weight (g), width (mm), length (mm) and yolk weight (g) remained non-significant among the groups. Shengyan *et al.* 2017 concluded that dietary betaine could improve HDEP and overall health of laying hens. This overall increase in egg production is may be due to reduction in stress and improvement of blood and gut profile due to betaine supplementations. The reduction in stress is may be achieved because of antioxidant and trans- methylation property of betaine.

Effect on semen quality: The effect of dietary betaine supplementation on semen quality is presented in Table 2. Semen concentration was found to be significantly ($P<0.01$) improved following betaine supplementation with highest increment in T4 treatment group as compared to T1 and T2 treatment groups, whereas, T3 treatment group showed an intermediate response. Mass motility (%) was found to be non-significant ($P>0.05$) among the treatments during 2nd and 6th week, whereas, it was found to be highest in T3 group at 4th week of experimental trial as compared to others. Semen quality was improved following betaine supplementation. Ezzat *et al.* 2011 reported that supplementation of betaine significantly increased the concentration and motility of spermatozoa in cocks, whereas in ducks they were comparable. Hassan *et al.* 2012 found that dietary supplementation of betaine significantly improved the sperm motility and total spermatozoa count compared to control under heat stress conditions in rabbit bucks.

Oxidative stress results from the imbalance between production of the reactive oxygen species (ROS) and the protective effect of the antioxidant system responsible for their neutralization and removal. An excess of ROS causes a pathological reaction resulting in damage to cells and tissues. Spermatozoa are particularly vulnerable to the harmful effects of ROS. Homocysteine produced during heat stress will have negative effect on sperm quality and quantity. The current results are consistent with previous studies in which homocysteine concentrations were reduced by dietary betaine supplementation in mice (Song *et al.* 2008). Betaine is a methyl donor compound involved in the conversion of homocysteine into methionine. This reaction mediated by betaine-homocysteine methyl-

transferase. Thus antioxidant and anti-homocysteine mechanism of betaine may be the probable reason for the increased concentration and motility of spermatozoa (Lugar *et al.* 2018).

Effect on hematological parameters: In females, significant ($P<0.05$) difference in RBC count, PCV, MCH and MCHC was observed among the treatments during 2nd week, whereas, at later periods they were comparable ($P>0.05$) among the groups (Table 3). In male birds, all hematological parameters were found non-significant ($P>0.05$) among the groups. Betaine supplementation did not exert any adverse effect on blood hematological parameters in broiler breeders. Gudev *et al.* 2011 reported that dietary supplementation of betaine at 0.7 g/kg had higher erythrocyte number than those fed 1.5 g/kg in laying hens. Awad *et al.* 2014 reported that hemoglobin concentration remained constant; whereas, RBC count was significantly improved following betaine supplementation in ducks. The finding suggests that there is no adverse effect of betaine on hematological parameters.

Effect on fertility, hatchability and embryonic mortality: Data related to effect of betaine supplementation on fertility, hatchability and total embryonic mortality in broiler breeders is summarized in Fig. 2. Fertility and Hatchability (TES: total eggs set and FES: fertile eggs set) was found to be higher in betaine supplemented groups as compared to control. This increase was highest in T4 group as compared to T1, T2 and T3 groups. Total embryonic death was decreased in betaine supplemented groups and found to be lowest in T4 treatment group followed by T3 and T2, groups. As we know, hatchability and fertility is very important parameter for breeders. In present study, dietary betaine supplementation improved fertility and hatchability and reduced total embryonic mortality in T4 group. The present study was in line with Heberle *et al.* 2016 who reported that breeder hens fed with 2 g betaine have higher fertility rate and embryonic survivability than 1 g and control groups. Cadogan *et al.* 2014 also reported that broiler breeders fed with 2 g significantly improved the hatchability of chicks. Awad *et al.* 2014 reported that hatchability (both TES: total eggs set and FES: fertile eggs set) was improved and total embryonic mortality was decreased with dietary supplementation of betaine at 0.5 and 1 g/kg of feed compared to control. The improved embryonic survival due to betaine might be due its osmoregulation and transmethylation properties. The reason for reduction in reproductive performance in summer is may be mutation in the enzyme methylene tetra hydro folate reductase (MTHFR), which increases homocysteine concentrations. Knockout mice deficient in the MTHFR enzyme have elevated homocysteine concentrations, oligospermia and infertility (Bezold *et al.* 2001). When betaine was supplemented to the mothers of MTHFR knockout mice during gestation and lactation, male offspring had a short-term improvement in fertility (Lugar *et al.* 2018).

Gholami *et al.* 2015 reported that increasing betaine

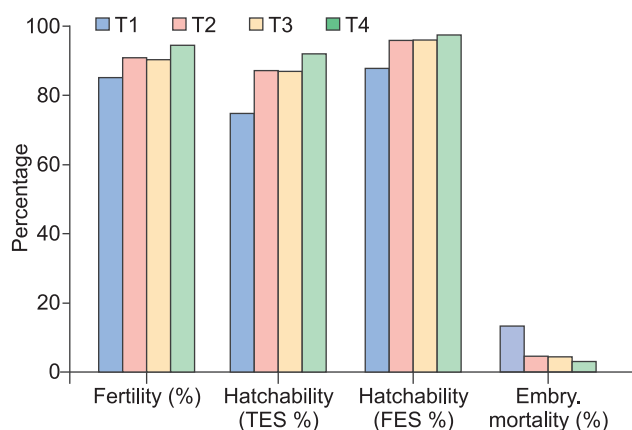


Fig. 2. Effect of betaine supplementation on fertility, hatchability and embryonic mortality in broiler breeders. T1, control group; T2, control+1 g betaine/kg diet; T3, control+2 g betaine/kg diet; T4, control+3 g betaine/kg diet. TES, Total eggs set; FES, Fertile eggs set.

supplementation increased fertility, hatchability (%), relative hatched chick weight and absolute chick weights. Cadogan *et al.* 2014 stated that high level of blood homocysteine reduces chick survivability which ultimately negatively affects the hatchery performance of breeders but betaine supplementation at 3 g/kg of feed significantly reduce the homocysteine concentration and increases the embryonic survivability and in turn hatchability (Van Wettere *et al.* 2012). Dietary betaine at 3 g/kg improved egg production, semen quality, fertility and hatchability (TES: total eggs set; FES: fertile eggs set) as well as reduced total embryonic mortality in broiler breeders. Thus, it can be concluded that, supplementation of betaine at 3 g/kg of diet was helpful in improving the reproductive performance as well as welfare in broiler breeders.

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REFERENCES

Anderson O S, Sant K E and Dolinoy D C. 2012. Nutrition and epigenetics: An interplay of dietary methyl donors, one-carbon metabolism and DNA Methylation. *Journal of Nutritional Biochemistry* **23**: 853–59.

Awad A L, Fahim H N, Ibrahim A F and Beshara M M. 2014. Effect of dietary betaine supplementation on productive and reproductive performance of Domyati ducks under summer conditions. *Egyptian Poultry Science* **34**: 453–74.

Bezold G, Lange M and Peter R U. 2001. Homozygous methylene tetrahydrofolate reductase C677T mutation and male infertility. *New England Journal of Medicine* **344**(15): 1172–73.

Burrows W H and Quinn J P. 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science* **16**: 19–24.

Cadogan D J, Wilson T, Morrisroe L and Scott P. 2014. *Betaine increases percentage hatched in broiler breeders*. Proceedings

of the 25th annual Australian Poultry Science Symposium New South Wales, Sydney, Australia: February 16–19.

Duncan D B. 1965. A Bayesian approach to multiple comparisons. *Technometrics* **7**(2): 171–222.

Esteve-Garcia E and Mack S. 2000. The effect of dl-methionine and betaine on growth performance and carcass characteristics in broilers. *Animal Feed Science and Technology* **87**: 85–93.

Ezzat W, Shoeib M S, Mousa S M M, Bealish A M A and Ibrahim Z A. 2011. Impact of betaine, vitamin C and folic acid supplementations to the diet on productive and reproductive performance of Matrouh poultry strain under Egyptian summer condition. *Egypt Poultry Science* **31**: 521–37.

Gholami J, Qotbi A A A, Seidavi A, Meluzzi A, Tavaniello S and Maiorano G. 2015. Effects of *in ovo* administration of betaine and choline on hatchability results, growth and carcass characteristics and immune response of broiler chickens. *Italian Journal of Animal Science* **14**: 187–92.

Gudev D, Popova-Ralcheva S, Yanchev I, Moneva P, Petkov E and Ignatova M. 2011. Effect of betaine on egg performance and some blood constituents in laying hens reared indoor under natural summer temperatures and varying levels of air ammonia. *Bulgarian Journal of Agricultural Science* **17**: 859–66.

Hasan T K and Abass D A. 2013. Effect of adding betaine to improving the productive performance of commercial layer hens at high temperature conditions in Iraq. *Diyala Agricultural Science Journal* **5**: 104–13.

Hassan R A, Morsy W A, Abd and El-Lateif A I. 2012. *Effect of dietary ascorbic acid and betaine supplementation on semen characteristics of rabbit bucks under high ambient temperature*. Proceedings of the 10th World Rabbit Congress (WRSA), Sharm El-Sheikh, Egypt. 273–277.

Heberle N, Edwards N, Cadogan D, Wilkinson S, Hughes R and Hynd P. 2016. *Effect of dietary natural betaine on broiler breeder hen performance and egg quality characteristics*. Proceedings of the Australian Society of Animal Production (ASAP), Adelaide.

ICAR. 2013. Nutrient requirements of poultry. New Delhi, India: *Indian Council of Agricultural Research*.

Lugar D W, Gellert T, Proctor J, Wilcock P, Richert B and Stewart K R. 2018. Effects of supplementation with betaine and superdosed phytase on semen characteristics of boars during and after mild heat stress. *The Professional Animal Scientist* **34**(4): 326–38.

Lukic M, Jokic Z, Petricevic V, Pavlovski Z, Skrbic Z and Stojanovic L. 2012. The effect of full substitution of supplemental methionine with betaine in broiler nutrition on production and slaughter results. *Journal of Animal Science and Biotechnology* **28**: 361–68.

Nawab A, Ibtisham F, Li G, Kieser B, Wu J, Liu W, Zhao Y, Nawab Y, Li K, Xiao M and An L. 2018. Heat stress in poultry production; Mitigation strategies to overcome the future challenges facing the global poultry industry. *Journal of Thermal Biology* **78**: 131–39.

Saeed M, Babazadeh D, Naveed M, Arain M A, Hassan F U and Chao S. 2017. Reconsidering betaine as a natural anti-heat stress agent in poultry industry: A review. *Tropical Animal Health and Production* **49**(7): 1329–38.

Shakeri M, Cottrell J, Wilkinson S, Ringuet M, Furness J and Dunshea F. 2018. Betaine and antioxidants improve growth performance, breast muscle development and ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. *Animals* **8**(10): 162.

- Shengyan H, Longsheng L, Guodong W, Xian G and Faming P. 2017. Effects of dietary betaine on production performance, egg quality and serum biochemical indexes of layers under heat stress condition. *Animal Husbandry and Feed Science* **9**: 80–85.
- Snedecor G W and Cochran W G. 1989. *Statistical methods*. 8th ed. Ames, USA: Iowa State University Press, Iowa.
- Song Z, Zhou Z, Deaciuc I, Chen T and McClain C J. 2008. Inhibition of adiponectin production by homocysteine: a potential mechanism for alcoholic liver disease. *Hepatology* **47**(3): 867–79.
- Rao S V R, Raju M V L N, Panda A K, Ria P S and Sunder, A G S. 2011. Effect of supplementing betaine on performance, carcass traits and immune responses in broiler chicken fed diets containing different concentrations of methionine. *Asian-Australasian Journal of Animal Science* **24**: 662–69.
- Van Wettere W H E J, Herde P. and Hughes P E. 2012. Supplementing sow gestation diets with betaine during summer increases litter size of sows with greater numbers of parities. *Animal Reproduction Science* **132**: 44–49.