



Effect of betaine supplementation on haematology, serum enzymes and hormone profile in gestating sows

ALOK MISHRA¹, A K VERMA², ASIT DAS³, PUTAN SINGH⁴ and N R SAHOO⁵

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

The study was conducted to study the effect of betaine supplementation on haematology, serum enzymes and hormones profile in gestating sows. For the study, artificially inseminated 18 crossbred (*Landrace* × *Desi*) sows were randomly distributed into 3 groups with 6 sows in each following a completely randomized design (CRD). Group T₀ was fed with basal diet (control) and betaine was supplemented in group T₁ and T₂ @ 3 g/kg basal diet during late pregnancy (d 76 onwards till parturition) and throughout the length of gestation, respectively. The results revealed that betaine supplementation had no significant effect on the haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, platelet count and white blood cells (WBC) count. Similarly, the serum enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and creatine kinase (CK) revealed no significant effect of betaine supplementation. The serum concentration of T₃ and T₄ hormone (ng/ml) of sows was significantly higher at the time of farrowing compared to the level at the time of insemination. The serum concentration of cortisol (ng/ml) of sows was significantly reduced in betaine supplemented groups compared to control. Thus, based on the results pertaining to cortisol levels in gestating, sows it can be concluded that betaine supplementation @ 3 g/kg diet, 76 day post insemination is beneficial to ameliorate the oxidative stress during pregnancy.

Key words: Betaine, Cortisol, Serum enzymes, Sows, Stress

India has the largest livestock population in the world that exceeds 512 million. Pig population in the country is 10.29 million, which contributes around 2.01% of the total livestock population (DADF 2014). Among the different livestock species, porcine production has high potential to contribute to high economic gain, particularly to the weaker sections of the society. This is because of high fecundity, high feed conversion efficiency, early maturing, short generation interval, and relatively small space requirement in pigs.

The periparturient period is a critical period in commercial sow herds. During the last third of gestation, the sow's metabolic and hormonal status changes dramatically (Weldon *et al.* 1994, Pere *et al.* 2000). Sows can become catabolic during the last month of gestation (Close *et al.* 1985), which was supported by increased concentrations of non-esterified fatty acids (NEFA). Pigs exhibit high plasma levels of cortisol and catecholamines in response to stress (D'Souza *et al.* 1998). Some studies showed oxidative stress effect of metallothionein in pigs (Li *et al.* 2007). Folate, vitamin B₁₂, methionine, choline, and betaine

are known methyl donors that can cause DNA methylation and histone methylation through altering single-carbon metabolism. Betaine (trimethyl glycine) is a quaternary ammonium compound (Yancey 1982). It is widely used as a feed additive in pig ration because it is chemically stable and non-toxic substance having osmo-protective and methyl donor characteristics.

Betaine has also been shown to protect cells from osmotic stress. Bagnasco *et al.* (1986) reported a high level of betaine in cells of the inner medulla of the kidney, which may indicate that betaine plays an important role in intracellular osmotic balance. Lever *et al.* (2004) reported that betaine is actively accumulated by many mammalian cells under hypertonic conditions. The present study hypothesized that response of sows to betaine supplementation strategy would depend on stage of gestation. Thus, the present study was conducted to optimize time of betaine supplementation in pregnant sows by assessing the haematology, serum enzymes, and serum hormone profile in gestating sows.

MATERIALS AND METHODS

Source of betaine: Betaine was purchased from Indian Trading Bureau Private Limited, Kolkata, West Bengal, India.

Experimental site, animals and housing: Eighteen adult

Present address: ¹PhD Scholar (dr.mishra04@gmail.com), ²Head and Director-CAFT (vermaak62@gmail.com), ^{3,4}Principal Scientist (drasitdas@rediffmail.com, putan60@gmail.com), Animal Nutrition Division; ⁵Senior Scientist (vet.nihar@gmail.com), Animal Genetics and Breeding Division.

healthy crossbred (*Landrace* × *Desi*) sows were selected from the Piggery farm (LPM section) of the institute. All the sows were ear tagged, and kept in well ventilated and clean pens under standard managerial conditions. The sows were vaccinated and dewormed for both endo- and ecto-parasites as per the schedule before the start of the experiment.

Experimental design and dietary treatment: Immediately after the artificial insemination, the sows were randomly allocated to three treatments (T_0 , T_1 and T_2) with six sows in each as per a completely randomized design (CRD). The

basal diet (conventional concentrate mixture) was formulated using crushed maize, wheat bran, deoiled soybean meal, mineral mixture, and common salt as per the specifications of NRC (1998) (Table 1). The treatment group (T_0) was fed basal diet and served as control. The basal diet was supplemented with betaine @ 3 g/kg diet and fed to treatment group T_1 (day 76 post insemination) and treatment group T_2 (whole gestation period).

Feeding schedule of the sows: All the experimental sows were offered weighed quantity of a mash feed as a single meal at 09:30 AM to meet their requirements (restricted feeding) with *ad lib.* fresh and clean drinking water. The mash feed was provided @ 2 kg/day/sow up to farrowing, @ 2.5 kg/day/sow up to 4 days post-farrowing, and thereafter @ 3.5 kg/day/sow up to weaning of piglets.

Blood collection: The blood samples from each experimental animal were collected before feeding and watering at 0 day and 114 day post insemination. The blood samples were collected in 2 separate vials, one for serum analysis and the second containing anticoagulant (EDTA) for haematology. The collected blood samples were kept at slanting position for 45 min, the serum was harvested and stored at -20°C till further analysis. The haematological parameters such as Hb, PCV, RBC, WBC, and platelet count were estimated by using Hematoanalyzer (Nihon Khoden, Japan). The estimation of serum enzymes such as ALT, AST, ALP, CK and LDH was done by using diagnostic kits

Table 1. Chemical and mineral composition of basal diet

Ingredient	Parts/100 kg
Crushed maize	55
De-oiled soybean meal	13
Wheat bran	30
Mineral mixture*	1.5
Common salt	0.5
Nutrient composition (As fed basis)	
DE (kcal/kg)**	3400
CP (%)***	15

*Composition of mineral mixture (% w/w): Ca, 24.79; P, 9.91; Mg, 0.87; Fe, 0.92; I, 0.078; Cu, 0.17; Mn, 0.22; Co, 0.02; Zn, 0.22; S, 2.04 and Se, 0.002. **Calculated values as fed basis.

***Analyzed values as fed basis.

Table 2. Effect of time of betaine supplementation on haematological profile in gestating sows

Treatment [†]	Days post-insemination		Mean	SEM	Statistical significance		
	0 d	114 d			T	P	T × P
<i>Hb (g/dl)</i>							
T_0	10.75	10.83	10.79	0.219	0.879	0.613	0.915
T_1	10.45	10.97	10.71				
T_2	10.45	10.57	10.51				
Mean	10.55	10.79					
<i>PCV (%)</i>							
T_0	37.5	37.17	37.33	0.334	0.676	0.755	0.842
T_1	37.83	38.17	38.00				
T_2	37.66	38.33	38.00				
Mean	37.67	37.89					
<i>RBC ($10^{12}/l$)</i>							
T_0	5.38	5.49	5.43	0.058	0.615	0.016	0.410
T_1	5.35	5.60	5.47				
T_2	5.33	5.80	5.56				
Mean	5.35 ^X	5.63 ^Y					
<i>Platelets ($10^9/l$)</i>							
T_0	357.0	348.3	352.7	4.784	0.533	0.027	0.474
T_1	361.0	341.8	351.4				
T_2	359.2	323.0	341.1				
Mean	359.1 ^Y	337.7 ^X					
<i>WBC ($10^9/l$)</i>							
T_0	12.68	12.74	12.71	0.085	0.444	0.987	0.608
T_1	12.61	12.37	12.49				
T_2	12.65	12.84	12.74				
Mean	12.65	12.65					

[†]Sow in control group (T_0), were fed with basal diet whereas diet of sow in group T_1 and T_2 were supplemented with 3 g/kg DM betaine either late gestation or whole gestation respectively. ^{X,Y}Means with different superscripts in a row differ significantly.

Table 3. Effect of time of betaine supplementation on serum enzyme profile in gestating sows

[†]Sow in control group (T₀), were fed with basal diet whereas diet of sow in group T₁ and T₂ were supplemented with 3 g/kg DM betaine either late gestation or whole gestation respectively. ^{X,Y}Means with different superscripts in a row differ significantly.

Table 4. Effect of time of betaine supplementation on hormone profile in gestating sow

Treatment [†]	Days post-insemination		Mean	SEM	Statistical significance		
	0 d	114 d			T	P	T × P
<i>T₃ (ng/ml)</i>							
T ₀	0.78	0.80	0.79	0.005	0.143	0.012	0.998
T ₁	0.78	0.81	0.80				
T ₂	0.78	0.80	0.80				
Mean	0.78 ^X	0.81 ^Y					
<i>T₄ (ng/ml)</i>							
T ₀	34.34	37.13	35.74	1.069	0.209	0.007	0.999
T ₁	34.50	37.37	35.94				
T ₂	34.34	37.33	35.84				
Mean	34.39 ^X	37.28 ^Y					
<i>Cortisol (ng/ml)</i>							
T ₀	33.40	40.32	36.86 ^B	1.087	0.001	0.150	0.026
T ₁	33.13	30.98	32.06 ^A				
T ₂	32.93	28.23	30.58 ^A				
Mean	33.15	33.17					

[†]Sows in control group (T₀), were fed with basal diet whereas diet of sow in group T₁ and T₂ were supplemented with 3 g/kg DM betaine either late gestation or whole gestation respectively. ^{X,Y}Means with different superscripts in a row differ significantly.

purchased from Coral Clinical System, Goa. The serum hormones such as T_3 , T_4 , and cortisol concentration in serum samples were determined using radio-immuno assay (RIA) kits supplied by Immunotech, France.

Statistical analysis: Each sow was used an experimental unit for data analysis. The data obtained from the study were subjected to two way analysis of variance by using general linear model procedure of SPSS software version 20. The treatment means were separated by Duncan's multiple range test (Duncan 1955) and the significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

There was no significant effect ($P > 0.05$) on Hb (g/dl), PCV (%), RBC ($10^{12}/l$), platelets ($10^9/l$) and WBC ($10^9/l$) following betaine supplementation (Table 2). The haematological and biochemical changes at different stage of gestation are important in monitoring the physiology and health status of the sow and also that of the developing foetus. The haematological parameters in this study were within the reference range for pigs (Carr 1998). However, an increased level of Hb and RBC at time of farrowing is attributed to more foetal development during the late gestation which creates great oxygen demand, which in turn stimulates the endocrine system to release more erythropoietin, the primary regulator of erythropoiesis in foetus and adults (Zanjani *et al.* 1974). Hytten (1985) and Guyton (2006) indicated that the red cell mass rises proportionately to the requirement of extra oxygen taken up in pregnancy and the bone marrow becomes increasingly active and produces extra red blood cells.

The serum AST, ALT, ALP, LDH, and CK values did not differ significantly and were within the reference range for pigs (Table 3). With regard to AST activity, our results were similar to those obtained by Reese *et al.* (1984). Hwang *et al.* (2010) also showed that dietary glycine betaine did not influence CPK levels in plasma.

The thyroid and adrenal gland are important for animals to regulate heat stress. The T_3 and T_4 are the main substances secreted by the thyroid. The cortisol is a steroid hormone synthesized by adrenal cortex cells, which regulate the metabolism of glucose, protein, fat, water and salt. Under stress, the cortisol level of body increases immediately which promotes the protein and fat catabolism, thus, increasing the body reserves for use to combat stress condition. The changes in the concentration of plasma cortisol have been used as an indicator of stress in pigs (Hicks *et al.* 1998, Sutherland *et al.* 2007, Song *et al.* 2011). The serum concentration of cortisol (ng/ml) was significantly ($P < 0.05$) lower in betaine supplemented groups (Table 4). This suggests that in betaine supplemented groups, stress level was significantly ($P < 0.05$) lower compared to non-supplemented group. Similarly, Matthews *et al.* (2001) reported that plasma cortisol decreased significantly ($P < 0.05$) following betaine supplementation under stress in swine. However, in contrast, Cabezon *et al.* (2016) and Cools *et al.* (2010) found that serum cortisol

concentrations were not influenced by dietary betaine supplementation in sows. Braganca *et al.* (1998) reported that, plasma cortisol concentrations were greater in sows fed *ad lib.* and housed at 20°C than sows fed *ad lib.* and housed at 30°C during lactation and after weaning. Whereas Parker *et al.* (2007) reported that betaine supplementation in steers during transportation of 24 to 48 h had no significant effect on the plasma cortisol level. Zhang *et al.* (2014) reported that plasma cortisol increased in the betaine supplemented groups in cows, which indicates that cows were affected by the heat during summer and it was explained by the fact that betaine lowers the basal metabolic rate. Schrama *et al.* (2003) reported that betaine reduces the maintenance requirement and metabolic heat production of growing pigs.

The results of the present study showed that the cortisol levels in sows decreased due to betaine supplementation and cortisol being a good indicator of stress, betaine supplementation (3 g/kg diet) 76 day post insemination is beneficial to ameliorate the oxidative stress during pregnancy.

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