



Effect of dietary betaine supplementation on production and reproductive performance, milk composition and serum antioxidant profile in gestating sows

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ABSTRACT

Present study was conducted to study the effect of betaine supplementation on production and reproductive performance, milk composition and serum antioxidant profile in gestating sows. For the study, 18 artificially inseminated crossbred (*Landrace* × *Desi*) sows were randomly distributed into three groups containing 6 sow each in completely randomized design (CRD). T₀ (control) group was supplemented with basal diet, whereas, T₁ and T₂ groups were fed basal diet supplemented with betaine @ 3 g/kg DM during late gestation (–76 days to farrowing) and throughout the length of gestation, respectively. Litter size at weaning was significantly increased in T₂ group as compared to control. Litter weight at weaning (kg) was significantly increased and weaning to estrus interval (days) was significantly decreased in T₁ and T₂ groups as compared to control. Serum superoxide dismutase level (ng/ml) was unaffected following betaine supplementation. Whereas, serum catalase level (ng/ml) and total antioxidant activity (Mmol/l) was significantly improved while malondialdehyde level (Mmol/l) was significantly reduced in betaine supplemented groups compared to control group. Thus, it can be concluded that, dietary betaine supplementation @ 3 g/kg throughout the length of gestation was helpful in improving reproduction performance, anti-oxidant defense as well as welfare of the pregnant sows.

Key words: Antioxidant profile, Betaine, Milk composition, Reproduction, Sows

Livestock are an important source of livelihood to rural population. Presently, India have the largest livestock population in world with 10.29 million pigs contributing around 2.01% of the total livestock population (DADF 2014). Due to high fecundity, high feed conversion efficiency, early maturation, short generation interval and relatively small space requirement, porcine production contributes high economic gain compared to other livestock enterprises.

It is noteworthy to mention that nutrients and bioactive food components can cause epigenetic changes and alter the expression of genes at the transcriptional levels. Betaine, also known as trimethylglycine, is a zwitterionic quaternary ammonium compound (Yancey 1982), that protect cells against osmotic inactivation (Craig 2004) and regulate gene expression through DNA methylation (Anderson *et al.* 2012). It is naturally present in whole grains, wheat bran (Westberg 1951), wheat (Chendrimada *et al.* 2002), spinach, marine organisms (Clarke *et al.* 1994) and sugar beets (de

Zwart *et al.* 2003).

Betaine functions as a methyl donor to increase methionine and decrease homocysteine concentration converting homocysteine to methionine (Mitchell *et al.* 1979). An increased blood homocysteine concentration, has been positively correlated to decreased conception rates in swine, which can be overcome by betaine supplementation (Matte *et al.* 2006). Many studies have indicated that supplementation of betaine improved growth performance, feed efficiency, pork quality (Smith *et al.* 1995) and decrease back fat thickness (Lawrence *et al.* 2002, Yu *et al.* 2004).

Available evidence suggests that fetal growth is most vulnerable to maternal dietary deficiencies of nutrients (protein and micronutrients) during the peri-implantation period and the period of rapid placental development (Sugden and Holness 2002, Waterland and Jirtle 2004). Hence, the present study hypothesized that response of maternal betaine supplementation on progeny would also depend on stage of gestation and attempted to identify precise time of methyl donor supplementation so as to develop supplementation strategy of betaine in pregnant sows. Thus, the present study was aimed to assess the effect of betaine supplementation on dry matter intake, body weight, reproductive parameters, milk composition and serum antioxidant profile in gestating sows.

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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 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 start of the experiment.

Experimental design and dietary treatments: Immediately after the artificial insemination, the sows were randomly allocated into three treatments (T_0 , T_1 and T_2) in a completely randomized design (CRD) consisting of six sows in each treatment. T_0 (control) group was fed with basal diet as per NRC (1998). Whereas, T_1 and T_2 groups were fed basal diet supplemented with betaine @ 3 g/kg DM during late gestation (–76 days to farrowing) and throughout the length of gestation, respectively.

Feeding 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.

***Feeding schedule:** Mash feed @ 2.0 kg/day/sow up to farrowing and @ 2.5 kg/day/sow up to 4 days post-farrowing. Thereafter, mash feed @ 3.5 kg/day/sow up to weaning.

Preparation of basal diet: The basal diet (conventional concentrate mixture) was prepared using crushed maize grain, wheat bran, deoiled soybean meal, mineral mixture and common salt (Table 1).

Feed intake and body weight: Weighed quantities of feed offered and residue collected were recorded on daily basis. Individual body weight of sows was recorded at monthly interval. Body weight of piglets at birth and at weaning was also recorded individually.

Reproductive performance: The reproductive performance of sows were analyzed in terms of litter size and litter weight (kg) of piglets at birth, average birth weight (kg) of piglets, litter size and litter weight (kg) of piglets at weaning, average weaning weight (kg) of piglets as well as weaning to estrus interval (days).

Blood collection: The blood samples from 6 sows per treatment were randomly collected on 0 day and 114 day post-insemination before feeding and watering, and serum was harvested as per the standard protocol.

Milk sample collection and analysis: Milk samples were collected from all sows at weaning (42 days) by full hand milking method. About 10 ml of milk samples were taken in sterile polypropylene tubes and immediately analysed for fat (%), protein (%), lactose (%), SNF (%) and specific gravity using Lactoscan.

Serum antioxidant profile: The antioxidant status of the sows for superoxide dismutase (SOD), catalase (CAT),

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.

malondialdehyde (MDA) levels and total antioxidant activity (TAA) were assessed at 0 and 114 days post-insemination. SOD and CAT levels in serum was assessed using Biont Assay ELISA kits; whereas, MDA level was assessed using ELISA kit manufactured and supplied by Chongqing Biospes Co. Ltd (China). TAA in serum was estimated as per the method described by Koracevic *et al.* (2001).

Statistical analysis: Data obtained from the study were subjected to analysis of variance. Treatment means were separated by Duncan's multiple range test (Duncan 1965) and was considered significant at $P < 0.05$. All analysis were performed using Statistical Package SPSS (v20.0)

RESULTS AND DISCUSSION

There was no significant effect ($P > 0.05$) on fortnight DMI (g/d) following betaine supplementation and was comparable among the groups (Table 2). Body weight of sows was recorded at 30th, 60th and 90th day post-insemination and no significant effect ($P > 0.05$) on body weight (kg) of sows was observed following betaine supplementation among the groups (Table 3). Similar finding was found by Albuquerque *et al.* (2017) in obese pig.

Table 2. Effect of dietary betaine supplementation on fortnight DMI (g/day) of sows

Fortnight	Treatment [†]			SEM	P value
	T_0	T_1	T_2		
1	1584	1585	1585	12.67	0.958
2	1625	1616	1650	13.05	0.583
3	1658	1663	1705	18.69	0.563
4	1686	1691	1725	12.70	0.433
5	1773	1790	1801	12.35	0.669
6	1870	1882	1896	17.82	0.412

[†]Sow in control group (T_0) was fed basal diet without betaine, whereas, sows in groups T_1 and T_2 were fed basal diet supplemented with betaine @ 3g/kg DM during late gestation (–76 days to farrowing) and throughout the length of gestation, respectively.

Table 3. Effect of dietary betaine supplementation on BW (kg) of sows

Days (post-insemination)	Treatment [†]			SEM	P value
	T ₀	T ₁	T ₂		
30	171.17	168.83	167.50	1.87	0.744
60	188.67	188.33	192.00	1.63	0.626
90	208.00	211.33	215.33	1.56	0.157

[†]Sow in control group (T₀) fed basal diet without betaine, whereas, sows in groups T₁ and T₂ were fed basal diet supplemented with betaine @ 3g/kg DM during late gestation (–76 days to farrowing) and throughout the length of gestation, respectively.

Litter size and weight (kg) at birth was comparable (P>0.05) among the groups (Table 4); whereas, litter size at weaning was significantly (P<0.05) increased in T₂ group as compared to T₀. Whereas, the T₁ group showed an intermediate response. Average birth and weaning weights (kg) were comparable (P>0.05) among the groups. Litter weight at weaning (kg) was significantly (P<0.01) higher in T₁ and T₂ groups as compared to control group. Weaning to estrus interval (days) was significantly (P<0.01) decreased in T₁ and T₂ groups as compared to control group with lowest weaning to estrus interval recorded in T₂ group. Ramis *et al.* (2011) reported that betaine supplementation 5 days before farrowing up to the end of lactation period reduced weaning to estrus interval, improved reproductive performance of gilts and sows as well as increased body weight gain of the piglets. van Wettter *et al.* (2012) reported that average live weight (LW) and LW gain of piglets were unaffected; whereas, total litter size increased following betaine supplementation to gestation sows. Litter size was affected by post-mating nutritional interventions altering conceptus survival (van Wettter *et al.* 2012). Literature suggests three possible mechanisms which support that

Table 4. Effect of dietary betaine supplementation on reproductive performance of sows

Parameter	Treatment [†]			SEM	P value
	T ₀	T ₁	T ₂		
Litter size at birth	7.3	8.5	9.3	0.38	0.091
Litter weight at birth (kg)	7.5	8.7	9.1	0.37	0.164
Average birth weight (kg)	1.01	1.02	0.99	0.02	0.888
Litter size at weaning	6.7 ^a	8.0 ^{ab}	9.0 ^b	0.35	0.014
Litter weight at weaning (kg)	54.0 ^a	65.9 ^b	73.0 ^b	2.63	0.004
Average weaning weight (kg)	8.20	8.26	8.12	0.12	0.896
Weaning to estrus interval (days)	9.17 ^c	6.83 ^b	5.25 ^a	0.42	0.001

Means bearing different superscript in a row differ significantly (P<0.05). [†]Sow in control group (T₀) was fed basal diet without betaine; whereas, sows in groups T₁ and T₂ were fed basal diet supplemented with betaine @ 3g/kg DM during late gestation (–76 days to farrowing) and throughout the length of gestation, respectively.

betaine supplementation could have increased conceptus survival, viz. reduced homocysteine concentrations due to altered methionine metabolism (Finkelstein 1990, van Wettter *et al.* 2012), increased efficiency of energy utilisation (Schrama *et al.* 2003), or increased secretion of growth hormones (Huang *et al.* 2007).

Milk fat (%), protein (%), lactose (%), solid not fat (SNF) (%) and specific gravity did not differ significantly and were comparable (P>0.05) among the groups (Table 5). Similar findings were reported by Ramis *et al.* (2011) who did not find any significant effect in milk component following dietary betaine supplementation to gestation sows.

Table 5. Effect of dietary betaine supplementation on milk component of sows

Parameter	Treatment [†]			SEM	P value
	T ₀	T ₁	T ₂		
Fat (%)	4.08	4.25	4.18	0.05	0.476
Protein (%)	4.18	4.20	4.19	0.01	0.458
Lactose (%)	6.36	6.39	6.47	0.02	0.196
SNF (%)	10.82	10.84	10.84	0.01	0.238
Specific gravity	0.772	0.773	0.770	0.01	0.785

[†]Sow in control group (T₀) was fed basal diet without betaine, whereas, sows in groups T₁ and T₂ were fed basal diet supplemented with betaine @ 3g/kg DM during late gestation (–76 days to farrowing) and throughout the length of gestation, respectively.

SOD level (ng/ml) in the serum was unaffected (P>0.05) following betaine supplementation (Table 6). Whereas, serum CAT level (ng/ml) (P<0.05) and TAA (Mmol/l) (P<0.001) was significantly improved as well as MDA level (Mmol/l) was significantly (P<0.01) reduced in betaine supplemented groups (T₁ and T₂) as compared to control group (T₀). An abnormality in the antioxidant defense system can increase the susceptibility of pigs to stress, resulting in decreased performance and reduced immune function. MDA is used as an indicator of lipid peroxidation (Nielsen *et al.* 1997) and an increased MDA level indicates cellular damage resulted from increased free radicals formation (Niedernhofer *et al.* 2003). Previous studies reported that the antioxidant capacity of betaine enabled it to scavenge free radicals and protect cells from oxidative damage in rats (Lu *et al.* 2008). Gheisari and Motamedi (2010) reported that dietary betaine supplementation reduced reactive oxygen species and free radicals formation, along with an improvement in the antioxidant defenses. Alirezaei *et al.* (2012) reported that glutathione peroxidase (GPx) and CAT activity were significantly higher and SOD activity was numerically higher in broilers with betaine supplemented groups compared to control group. It has been reported that the three methyl groups of betaine plays a crucial role in its antioxidant activity and it also enhances the non-enzymatic antioxidant defenses via the methionine-homocysteine cycle to form a protective membrane around cells (Zhang *et al.* 2016).

Table 6. Effect of dietary betaine supplementation on serum antioxidant profile in gestating sows

Days post-insemination			Treatment mean	P value		
Treatment [†]	0 day	114 day		T	P	T×P
<i>SOD (ng/ml)</i>						
T ₀	6.63± 0.88	5.65±0.30	6.14±0.47	0.129	0.266	0.020
T ₁	6.07± 0.54	7.72±0.22	6.89±0.37			
T ₂	6.33±0.57	8.03±0.21	7.18±0.39			
Period mean	6.34± 0.37	7.13±0.29				
<i>Catalase (ng/ml)</i>						
T ₀	7.43±0.24	7.20±0.18	7.32 ^A ±0.15	0.037	0.266	0.044
T ₁	7.72±0.22	7.60±0.44	7.66 ^{AB} ±0.23			
T ₂	7.53±0.40	8.72±0.21	8.13 ^B ±0.28			
Period mean	7.56±0.16	7.84±0.23				
<i>MDA (Mmol/l)</i>						
T ₀	6.24±0.27	6.79±0.07	6.52 ^B ±0.16	0.001	0.008	0.001
T ₁	6.25±0.27	5.53±0.21	5.89 ^A ±0.20			
T ₂	6.27±0.22	4.97±0.17	5.62 ^A ±0.24			
Period mean	6.26 ^Y ±0.14	5.76 ^X ±0.20				
<i>TAA (Mmol/l)</i>						
T ₀	1.15± 0.10	1.01± 0.07	1.08 ^A ±0.06	0.001	0.001	0.001
T ₁	1.13±0.05	2.01±0.21	1.57 ^B ±0.17			
T ₂	1.11±0.05	2.15±0.24	1.63 ^B ±0.20			
Period mean	1.13 ^X ±0.04	1.72 ^Y ±0.16				

Means bearing different superscripts in a row differ significantly (P≤0.05) and (P≤0.01). Means bearing different superscripts in a column differ significantly (P≤0.05) and (P≤0.01). [†]Sow in control group (T₀) was fed basal diet without betaine, whereas, sows in groups T₁ and T₂ were fed basal diet supplemented with betaine @ 3g/kg DM during late gestation (–76 days to farrowing) and throughout the length of gestation, respectively.

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