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Effect of Maternal Betaine Supplementation on Growth, Plane of Nutrition, Blood Biochemical Profile and Antioxidant Status of Progeny Pigs

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ABSTRACT

Background: Dietary supplementation of methyl donors like vitamins B_9, B_{12}, choline and betaine have been reported to reduce oxidative stress not only in sows but can also reduce oxidative stress in offspring through epigenetic modulation of DNA. However, cell proliferation and fetal development and oxidative stress associated with it is not uniform during the whole length of gestation. Hence this experiment was conducted to study the effects of maternal betaine supplementation on growth, plane of nutrition and antioxidant profile of progeny pigs.

Methods: Eighteen crossbred (Landrace × Desi) sows were randomly distributed into three groups of six each in an experiment based on completely randomized design (CRD). The sows in control (T_0) were fed standard ration to meet their requirements. Supplementary betaine at 3 g/kg DM were provided either during late pregnancy (d 76 onwards till parturition) only or throughout the length of gestation to the sows of groups T_1 and T_2, respectively. The samples of feed offered, residue and faeces were analyzed for proximate principles following the standard procedures. Blood samples from the progeny piglets were collected and antioxidant status of the piglets assessed by the measurement of superoxide dismutase (SOD) and catalase (CAT) by using standard kits.

Result: The serum concentration of SOD was comparable ($p>0.05$) among the groups, whereas serum concentration of catalase was higher ($p<0.05$) in piglets born to the dam exposed to supplementary beanie during gestation, the best response was observed whilst betaine was supplemented in the maternal diets during the whole length of gestation. It was concluded that supplementation of betaine at 3 g/kg in the diet of pregnant sows improved the antioxidant capacity of piglets borne to them.

Key words: Antioxidant profile, Blood biochemical, Maternal betaine, Piglet growth.

INTRODUCTION

Reactive oxygen species (ROS), including superoxide, hydrogen peroxide and hydroxyl radical are cytotoxic agents that lead to significant oxidative damage. High levels of ROS during embryonic, fetal and placental development are a feature of pregnancy (Kais *et al*., 2010). Consequently, an imbalance between antioxidants and ROS production has been reported as a key factor in early fetal loss (Hempstock *et al.,* 2003) and is responsible for affecting female reproductive processes (Agarwal and Allamaneni, 2004). Additionally, oxidative damage causes abnormal intrauterine development of an embryo and may increase the risk of developing metabolic diseases later in life (Taylor and Poston, 2007).

Lin *et al.* (2012) reported that an improvement in maternal, placental and fetal antioxidant capacities benefit fetal development and growth. Thus, fetal development and growth processes are susceptible to oxidative stress and may continuously affect the offspring, whereas nutritional factors may contribute to adverse pregnancy outcomes and increase the susceptibility of offspring to diseases. Homocysteine, a thiol-containing derivative of methionine metabolism, undergoes auto-oxidation *in vivo* and generates ROS which causes vascular endothelial damage (Szasz *et al.,* 2007). High maternal homocysteine concentration has been associated with low birth weight in offspring, preeclampsia, preterm birth and intrauterine fetal death (Murphy *et al.,* 2004; Tamura and Picciano, 2006). The dietary supplementation of vitamins B_9, B_{12}, choline and betaine have been reported to reduce homocysteine levels by reducing oxidative stress in sows (Atkinson *et al.,* 2009; Van-Wettere *et al.,* 2013). Betaine is a quaternary ammonium compound (Yancy *et al.,* 1982; Lokhande *et al*., 2019) and used widely as a feed additive in pig ration because it is a chemically stable and non-toxic substance having osmo-protective and methyl donor characteristics. Maternal nutrition affects metabolic homeostasis in the offspring of most animals (Grove and Smith, 2003) but it is evident from the literature that maternal supplementation of

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betaine can cause epigenetic changes in offspring (Bestor, 2000). However, it was hypothesized that the progeny antioxidant status in response to maternal betaine supplementation would also depend on the duration of betaine supplementation during pregnancy. Thus, the objective of the present study was to assess the response of progeny to the dietary betaine supplementation of sows in terms of growth, nutritional and antioxidant status.

MATERIALS AND METHODS

Source of betaine

Betaine was purchased from Indian Trading Bureau Private Limited, Kolkata, West Bengal, India.

Experimental animals and housing

Eighteen adults healthy crossbred (*Landrace* × *Desi*) sows of same age reared under similar conditions were selected from the Piggery Farm, LPM Section, ICAR-IVRI, Izatnagar for the present investigation. This experiment was during November, 2017 and June, 2018. All the sows were ear-tagged and kept in well ventilated and clean pens under standard managemental conditions. The sows were vaccinated and dewormed for both endo- and ectoparasites as per the schedule before the start of the experiment.

Experimental design and dietary treatments

Immediately after the artificial insemination, the sows were divided into three groups (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 group T_0 fed basal diet served as control. The basal diet was supplemented with betaine @ 3 g/kg diet and fed to group T_1 during the second half of the gestation period and group T_2 during the whole gestation period. Betaine was purchased from Indian Trading Bureau Private Limited, Kolkata West Bengal, India.

Feeding schedule of the sows

The sows were offered weighed quantity of a mash feed as a single meal at 09:30 AM to meet the nutrient requirements (restricted feeding) with *ad lib* fresh and clean drinking water. The mash feed was provided @ 2.0 kg/day/sow up to farrowing, 2.5 kg/day/sow from farrowing to 4 days post-farrowing and 3.5 kg/day/sow thereafter till the weaning of piglets.

Measurement and analysis

The individual body weights of progeny piglets were recorded at weekly intervals to arrive at mean weekly body weight under each treatment group. The samples of feed offered, residue and faeces were analyzed for proximate principles following the standard procedures (AOAC, 2000) to arrive at dry matter intake (DMI), protein intake, energy intake and nutritive value of feed under respective treatment groups. The blood samples from the progeny piglets of each experimental group were collected before feeding and watering at 90 days (grower phase) and 180 days (finisher phase) of age in commercially available clot activating tube. The tubes were kept in a slanting position for 30-40 minutes for clot formation. The tubes were then centrifuged at 1500 rpm for 10 minutes in a refrigerated centrifuge. The harvested serum was subjected to the analysis of serum glucose total protein, albumin, creatinine, cholesterol and triglyceride using commercial diagnostic kits of Coral Clinical Systems, Tulip Diagnostics, India.

The resulting supernatants were transferred into a clean polypropylene tube with the help of Pasteur pipette and stored under cold conditions (-20°C) until further analysis. The antioxidant status of the piglets assessed by the measurement of SOD and catalase (CAT) levels by using Cayman Diagnostic Biont Assay ELISA Kit which was based on the biotin double antibody sandwich technology.

Statistical analysis

Data obtained from the study were subjected to analysis of variance. Treatment means were separated by Duncan's multiple range test (Duncan, 1955) and was considered significant at $P<0.05$. All analysis were performed using statistical package SPSS (20.0).

RESULTS AND DISCUSSION

There was no significant difference among the groups in weekly body weight changes in pigs during the whole experimental period (Fig 1). Similar findings were found by Cai *et al.* (2014) where maternal betaine supplementation

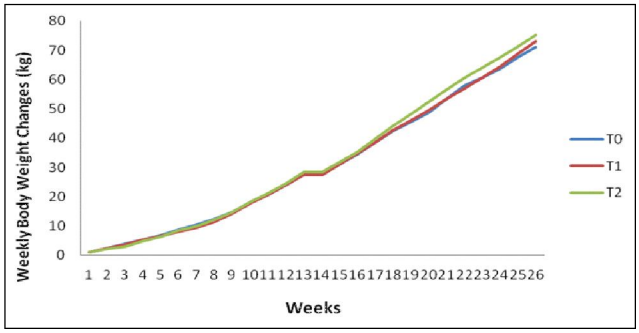

Fig 1: Weekly body weight changes among groups.

Table 1: Physical composition of diets for piglets.

Ingredients	Body weights (kg)			
(Parts/100 kg)	5-10	10-20	20-50	50-80
Crushed maize	39.5	44.5	49	52
Deoiled soya bean meal	38.5	30	23	14.5
Wheat bran	20	23.5	26	31.5
Mineral mixture*	1.5	1.5	1.5	1.5
Common salt	0.5	0.5	0.5	0.5
CP (%)	23.7	20.8	18.4	15.6
DE (kcal/kg)	3400	3400	3400	3390

*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.

Table 2: Effect of time of maternal betaine supplementation on nutritive value and plane of nutrition in pigs during grower phase.

Attributes	Dietary treatments*			SEM	P value
	T_0	T_1	T_2		
Average body weight					
kg	42.5±2.35	43.1±2.78	43.57±1.38	1.224	0.945
$kgW^{0.75}$	16.62±0.69	16.79±0.82	16.95±0.40	0.359	0.940
Dry matter intake					
g/d	1518±35	1589±22	1615±32	19.44	0.101
g/kg $kgW^{0.75}$	92.02±3.83	95.81±4.95	95.63±3.18	2.241	0.760
Crude protein intake					
g/d	250.53±5.89	262.21±3.69	266.61±5.38	3.210	0.101
g/kg $kgW^{0.75}$	15.18±0.63	15.81±0.82	15.78±0.52	0.370	0.760
Digestible crude protein intake					
g/d	186.42±8.02	197.21±3.37	204.43±5.92	3.750	0.142
$g/kgW^{0.75}$	11.30±0.63	11.92±0.74	12.09±0.43	0.344	0.642
Energy utilization (kcal/d)					
GE intake	7981.37±51.02	8038.37±71.53	8061.03±85.95	39.33	0.721
DE intake	6417.90±47.67	6482.20±62.08	6503.53±71.62	34.37	0.599
ME intake	6161.18±45.76	6222.91±59.60	6243.39±68.76	33.00	0.599
Nutritive value (%)					
DCP	12.25±0.28	12.42±0.23	12.65±0.24	0.140	0.541
TDN	69.45±1.79	69.28±0.56	70.07±0.35	0.603	0.870

GE-Gross energy; DE-Digestible energy; ME-Metabolizable energy; DCP-Digestible crude protein; TDN-Total digestible nutrients.
* T_0 Piglets from sow of control group. T_1 and T_2 Piglets from sow supplemented with @3 g/kg DM betaine either late gestation or whole gestation respectively.

Table 3: Effect of time of maternal betaine supplementation on nutritive value and plane of nutrition in pigs during finisher phase.

Attributes	Dietary treatments*			SEM	P value
	T_0	T_1	T_2		
Average body weight					
kg	71.18±2.78	73.15±3.48	75.28±2.31	1.621	0.615
$kgW^{0.75}$	24.49±0.71	24.99±0.90	25.55±0.59	0.416	0.612
DMI					
g/d	2155±26.94	2214±46.86	2222±29.63	20.61	0.374
$g/kgW^{0.75}$	88.36±2.64	89.03±2.85	87.26±2.67	1.486	0.898
Crude protein intake					
g/d	329.9±4.12	339.0±7.18	340.2±4.54	3.155	0.374
$g/kgW^{0.75}$	13.53±0.40	13.63±0.44	13.36±0.41	0.277	0.898
Digestible crude protein intake					
g/d	262.8±5.82	275.1±7.26	282.3±5.69	3.930	0.121
$g/kgW^{0.75}$	10.77±0.37	11.06±0.40	11.09±0.38	0.211	0.819
Energy utilization (kcal/d)					
GE intake	9868±282.42	10195±164.11	10213±160.05	120.2	0.444
DE intake	8381±270.01	8673±148.16	8694±147.65	112.4	0.472
ME intake	8045±259.21	8326±142.23	8346±141.74	170.9	0.472
Nutritive value (%)					
DCP	12.20±0.24	12.42±0.10	12.70±0.11	0.100	0.122
TDN	78.86±1.06	78.36±1.08	79.74±0.50	0.499	0.871

GE-gGross energy; DE-Digestible energy; ME-Metabolic energy; DCP-Digestible crude protein; TDN-Total digestible nutrient.
* T_0 Piglets from sow of control group. T_1 and T_2 Piglets from sow supplemented with @3 g/kg DM betaine either late gestation or whole gestation respectively.

did not affect the body weight of piglets, whereas Liu *et al.* (2017) showed that maternal methyl donor supplementation during gestation affected average body weight of piglets and was not significantly differed from that in the methyl donor group at birth, but tended to be lower at weaning. The variation in response could be due to level of inclusion (Eklund *et al*., 2005) and the ratio of lysine: calorie in the basal diet (Matthews *et al*., 1998).

Average body weight (kg), DMI (g/d) and (/kg$W^{0.75}$), CPI (g/d) and (g/kg$W^{0.75}$), energy utilization (GE intake, DE intake, ME intake) and nutritive value (%DCP andTDN) were comparable among the groups (Table 2 and 3). Similar findings were observed by Schrama *et al.* (2003), where they studied the effect of betaine supplementation on energy metabolism in growing pigs. It had earlier been reported that due to its osmolytic properties; betaine can reduce

Table 4: Effect of time of maternal betaine supplementation on blood biochemical profile of pigs.

Treatments	90 d	180 d	Mean	SEM	Significance		
					T	P	T*P
Serum glucose (mg/dl)							
T_0	89.92±0.34	89.92±0.34	89.92±0.23	0.131	0.959	0.876	0.994
T_1	90.0±0.36	89.93±0.32	89.97±0.23				
T_2	90.05±0.36	89.98±0.35	90.02±0.24				
Mean	89.99±0.19	89.94±0.18					
Albumin (g/dl)							
T_0	3.35±0.12	3.38±0.11	3.37±0.08	0.039	0.983	0.604	0.983
T_1	3.32±0.09	3.38±0.07	3.35±0.06				
T_2	3.35±0.12	3.38±0.11	3.37±0.08				
Mean	3.34±0.06	3.38±0.05					
Globulin (g/dl)							
T_0	2.55±0.11	2.53±0.10	2.54±0.07	0.032	0.796	0.981	0.963
T_1	2.58±0.07	2.59±0.08	2.58±0.05				
T_2	2.58±0.07	2.60±0.06	2.59±0.04				
Mean	2.57±0.05	2.57±0.04					
Total protein (g/dl)							
T_0	5.90±0.17	5.91±0.15	5.90±0.11	0.053	0.933	0.692	0.120
T_1	5.89±0.13	5.98±0.12	5.93±0.09				
T_2	5.93±0.14	5.98±0.13	5.96±0.09				
Mean	5.91±0.08	5.95±0.07					
A/G ratio							
T_0	1.32±0.07	1.35±0.07	1.34±0.05	0.021	0.730	0.980	0.730
T_1	1.29±0.05	1.31±0.04	1.30±0.03				
T_2	1.30±0.06	1.31±0.05	1.30±0.04				
Mean	1.31±0.03	1.32±0.03					
Creatinine (mg/dl)							
T_0	1.06±0.05	1.12±0.05	1.09±0.04	0.023	0.583	0.185	0.992
T_1	1.1±0.06	1.16±0.04	1.13±0.04				
T_2	1.11±0.08	1.18±0.05	1.15±0.04				
Mean	1.09±0.04	1.15±0.03					
Cholesterol (mg/dl)							
T_0	54.42±0.47	53.93±0.21	54.18±0.25	0.179	0.351	0.110	0.397
T_1	54.23±0.37	53.53±0.20	53.88±0.23				
T_2	54.35±0.42	52.82±0.59	53.58±0.42				
Mean	54.33±0.43	53.43±0.24					
Triglyceride (mg/dl)							
T_0	16.58±0.33	16.18±1.1	16.38±0.60	0.277	0.092	0.175	0.074
T_1	15.53±0.35	15.72±0.15	15.63±0.33				
T_2	15.40±0.27	15.87±0.20	15.63±0.28				
Mean	15.83±0.17	15.92±0.53					

* T_0 Piglets from sow of control group. T_1 and T_2 Piglets from sow supplemented with @3 g/kg DM betaine either late gestation or whole gestation respectively.

Table 5: Effect of time of maternal betaine supplementation on serum antioxidant profile in pigs.

*Treatments	90 d	180 d	Mean	SEM	Significance		
					T	P	T*P
		SOD (ng/ml)					
T_0	6.63±0.88	6.15±0.63	6.39±0.52				
T_1	6.85±0.78	6.67±0.67	6.76±0.49	0.289	0.806	0.669	0.969
T_2	6.93±0.75	6.80±0.80	6.87±0.52				
Mean	6.81±0.44	6.54±0.39					
		CAT (ng/ml)					
T_0	5.14±0.11	4.65±0.26	4.89^{A}±0.15	0.064	0.039	0.041	0.301
T_1	5.23±0.12	5.08±0.09	5.15^{AB}±0.07				
T_2	5.30±0.07	5.22±0.09	5.26^{B}±0.06				
Mean	5.22^{Y}±0.06	4.98^{X}±0.11					

SOD: Superoxide Dismutase, CAT: Catalase.

* T_0 Piglets from sow of control group. T_1 and T_2 Piglets from sow supplemented with @3 g/kg DM betaine either late gestation or whole gestation respectively.

$^{A,B/X,Y}$Means with different superscripts in a column/row differ significantly.

energy expenditure for the ion pump, particularly in the cells of the gastrointestinal tract (Simon, 1999). However, in this experiment, betaine supplementation in maternal diet during gestation showed no influence on energy utilization in piglets during the grower/finisher phase. The discrepancy in the response could be attributed to level of inclusion (Eklund *et al.,* 2005) and dietary lysine: calorie ratio (Matthews *et al.,* 1998). DMI, dry matter intake; CPI, crude protein intake; DCPI, digestible crude protein intake; GE, gross energy; DE, digestible energy; ME, metabolic energy; DCP, digestible crude protein; TDN, total digestible nutrient.

There was no significant difference in serum concentration of glucose (mg/dl), albumin (g/dl), globulin (g/dl), total protein (g/dl), A/G ratio, creatinine (mg/dl), cholesterol (mg/dl) and triglyceride (mg/dl) among the groups (Table 4). All the values were within normal range reported for pigs. Thus, it is evident that maternal supplementation of betaine during gestation showed no adverse effect on general metabolism of the progeny at later stages of life. This corroborates well with the findings of Cai *et al*. (2014).

The serum concentration of SOD (ng/ml) was comparable ($p>0.05$) among the groups, whereas serum concentration of catalase (ng/ml) was significantly higher ($p<0.05$) in piglets born to dam exposed to supplementary betaine during gestation (Table 5). Similarly, supplementation of betaine in broiler diets improved the antioxidant status (Jamina *et al*., 2020). This finding is also in accordance with that of Mou *et al*. (2018), where they reported that supplementation with methyl donors increased the antioxidant capacity of sows as well as newborn piglets by increasing the activities of catalase and glutathione peroxidase. In another study, supplementation of antioxidant improved the antioxidant status and the ability of pigs to combat stress (Chakraborty *et al*., 2018). Homocysteine (Hcy) concentrations in pigs are typically several folds higher than in other species, which results in increased levels of ROS. The betaine-homocysteine S-methyltransferase (BHMT) enzyme helps in the conversion of Hcy to Methionine by facilitating the transfer of methyl group from betaine.

The maternal nutrition might reduce oxidative stress in the placenta and thus affect the redox status of the offspring, which is in agreement with the results of Lin *et al.* (2012), who reported that diets supplemented with methyl donors appear to decrease the homocysteine concentration in sows by the remethylation or trans-sulfuration pathways and increase maternal and offspring antioxidant capacity. This is in agreement with previous results; the present study showed that prepartum supplementation with betaine improved the antioxidant capacity of piglets.

CONCLUSION

In conclusion, the findings of the present study showed that dietary betaine supplementation @ 3 g/kg throughout the length of gestation reduced oxidative stress and improved the antioxidant capacity of piglets.

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