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SHORT COMMUNICATION

Effect of Betaine Supplementation on Serum Metabolite Profile in Gestating Sows

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ABSTRACT

Present study was conducted to investigate the effect of betaine supplementation on serum metabolite profile in gestating sows. For the study, eighteen crossbred (*Landrace X Desi*) inseminated sows were randomly distributed into three treatment groups with six sows per treatment in completely randomized design. Animals in T_0 (control) group were fed basal diet, whereas, T_1 and T_2 treatment groups were fed basal diet supplemented with betaine @ 3g/kg dry matter either during late gestation (76 days before to farrowing) or throughout the length of gestation, respectively.There were no significant (P>0.05) differences in serum glucose, albumin, globulin, total protein, albumin/globulin ratio and creatinine levels among the groups. whereas, serum cholesterol and triglyceride levels were significantly(P<0.05) lower in T_2 group as compared to T_0 and T_1 groups. Thus, it can be concluded that, dietary betaine supplementation @ 3 g/kg throughout the length of gestation was helpful in decreasing the serum cholesterol and triglyceride levels in sows.

Key words: Betaine, Cholesterol, Gestating sows, Metabolicprofile, Triglyceride

Betaine is a by-product of the sugar beet processing, and chemically a trimethyl derivative of the amino acid glycine. It is commercially available as a feed additive for livestock. Betaine provides three methyl groups which can be used in transmethylation reactions for the synthesis of numerous substances. The dual function of betaine as methyl donor and amino acid gets it involved in protein and energy metabolism Eklund *et al.* (2005). Moreover, under stress situation or disease, methylation reactions are needed for building the immune defense mechanism as well the synthesis of polyamides, which play a role in tissue repair process. Betaine supplementation had shown positive effects regarding mortality reduction under heat stress conditions, improved immunity and the health performance of birds (Khattak *et al.,* 2012; Attia *et al.,* 2016).

Betaine plays an important role in lipid metabolism and improves meat production (Akhavansalamat and Ghasemi 2016). Various studies suggest that lipid metabolism is also affected by betaine (Hanczakowska *et al.,* 1999; Huang *et al.,* 2008; Rojas-Cano *et al.,* 2011; Sales, 2011; Yang *et al.,* 2009), which is considered to be a lipotropic compound. Betaine indirectly stimulates the synthesis of carnitine necessary for the transport of long-chain fatty acids to mitochondria, where they are oxidized (Zabaras-Krick, 1997). According to some authors, betaine enables decreasing the amount of adipose tissue through the stimulation of fatty acid oxidation and contribution to the synthesis of carnitine (Saunderson and McKinlay, 1990); Nakev *et al.,* 2009). Fernandez-Figares *et al.* (2002) observed that betaine can reduces carcass fat concentration in pigs and at the same time it reveals that the addition of 0.5% betaine has no impact on the oxidation and utilization of palmitic acid. This suggests that the effect of betaine on lipid metabolism could be related to mechanisms other than oxidation (Wray-Cahen *et al.,* 2004). Loest *et al.* (2002) found that betaine may decrease the demand for choline methyl groups, thus increasing choline availability for lipid metabolism. This was supported by the findings of

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Yao and Vance (1989). Thus, the present study was carried out to assess the effect of betaine supplementation on serum metabolite profile in gestating sows.

The experimental procedures carried out in the study were approved by the Institutional Animal Ethics Committee. Following a completely randomized design, eighteen healthy crossbred (*Landrace X Desi*) sows, freshly inseminated, were divides in to three groups with six animals in each. The three groups of sows were assigned to three treatment *viz.* T_0 (basal diet), T_1 (basal diet + 3g betaine/kg diet from 76 day of pregnancy to furrowing), and T_2 (basal diet + 3g betaine/kg diet for whole gestation period). The betaine was purchased from Indian Trading Bureau Private Limited, Kolkata, West Bengal, India. The basal diets were formulated as per the recommendations of NRC (1998) (Table 1). The basal feed was provided @ 2.0 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 with *ad lib* fresh and clean drinking water.

The blood samples from six sows per treatment were collected on 0 day and 114 day post-insemination before feeding and watering in commercially available clot activating tubes to harvest the serum. 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 data obtained from the study were subjected to two way analysis of variance. Treatment means were separated by Duncan's multiple range test (Duncan, 1955) and were considered significant at P<0.05. All analysis were performed using statistical software package SPSS version 20.0.

Table 1. Chemical and nutrient composition of basal diet

Ingredients	Parts/100 kg
Crushed maize	55
Deoiled 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

The effect of dietary betaine supplementation on serum metabolites studied during experimental trial is presented in Table 2. There were no significant differences in serum concentrations of glucose (mg/dl), albumin (g/dl), globulin (g/dl), total protein (g/dl), A/G ratio and creatinine (mg/dl) among the groups following betaine supplementation. However, concentrations of cholesterol (mg/dl) and triglyceride (mg/dl) differed significantly among the groups; values being lower in group T_2 as compared to T_0 and T_1 groups (Table 2). The efficacy of betaine in regulating the concentration of cholesterol in pigs shows variable results and seems to depend on both animal and dietary factors. Although the results were inconsistent, but it seems that betaine might affect cholesterol partitioning or enhances the transport of cholesterol. Similarly, Hwang *et al.* (2010) and Fernandez *et al.* (2009) reported that oral supplementation of betaine (4 g/kg) for 15 days before parturition up to lactation in goats resulted in lower triglycerides level than the control group. The supplementation of betaine at 0.2% in sow diet resulted in lower serum concentration of total cholesterol as compared to control (Yang *et al.,* 2009). However, major involvement of betaine in lipid metabolism was in its lipotropic activity. Barak *et al.* (1994) reported that the dietary betaine has been shown to stimulate liver lipid mobilization and alter the blood lipoprotein profile. The natural lipids are mainly composed of triglycerides, which form the principal part of depot lipids in meat systems (Fernandez *et al.,* 1998). Li *et al.* (2017) showed that betaine supplementation decreased the concentration of serum cholesterol and HDL-cholesterol and increased cholesterol level in muscle, which was consistent with

Table 2 Effect of time of betaine supplementation on serum metabolite profile in gestating sows

Treatment†	Days post-insemination		Mean	SEM	Significance		
	0 d	114 d			T	P	T*P
Serum glucose (mg/dl)							
T_0	77.80±1.89	79.03±1.12	78.42±1.06	0.833	0.055	0.378	0.980
T_1	77.88±2.89	79.87±1.71	78.88±1.63				
T_2	79.02±2.47	79.08±2.50	79.05±1.68				
Mean	78.23±1.34	79.33±1.02					
Albumin (g/dl)							
T_0	4.44±0.11	4.27±0.08	4.35±0.07	0.034	0.871	0.049	0.951
T_1	4.40±0.10	4.28±0.08	4.34±0.06				
T_2	4.45±0.06	4.32±0.07	4.38±0.05				
Mean	4.43±0.05	4.29±0.04					
Globulin (g/dl)							
T_0	2.80±0.14	2.57±0.05	2.68±0.08	0.034	0.265	0.047	0.069
T_1	2.75±0.06	2.52±0.05	2.63±0.05				
T_2	2.52±0.05	2.60±0.06	2.56±0.04				
Mean	2.69^{Y}±0.06	2.56^{X}±0.03					
Total protein (g/dl)							
T_0	7.24±0.08	6.83±0.10	7.03±0.08	0.043	0.580	0.001	0.120
T_1	7.15±0.08	6.80±0.08	6.98±0.08				
T_2	6.97±0.10	6.92±0.10	6.94±0.07				
Mean	7.12^{Y}±0.05	6.85^{X}±0.05					
A/G Ratio							
T_0	1.61±0.11	1.67±0.05	1.64±0.06	0.025	0.403	0.728	0.225
T_1	1.61±0.06	1.71±0.05	1.66±0.04				
T_2	1.77±0.02	1.67±0.04	1.72±0.03				
Mean	1.66±0.04	1.68±0.02					
Creatinine (mg/dl)							
T_0	1.31±0.10	1.33±0.10	1.32±0.07	0.032	0.453	0.078	0.555
T_1	1.31±0.06	1.46±0.06	1.38±0.04				
T_2	1.33±0.08	1.50±0.07	1.41±0.06				
Mean	1.31±0.04	1.43±0.05					
Cholesterol (mg/dl)							
T_0	54.42±0.70	47.58±1.18	51.00^{B}±1.22	0.854	0.008	0.001	0.057
T_1	54.48±0.46	45.27±0.68	49.88^{AB}±1.44				
T_2	53.92±0.94	42.82±0.54	48.37^{A}±1.75				
Mean	54.27^{Y}±0.40	45.22^{X}±0.66					
Triglyceride (mg/dl)							
T_0	92.41±0.42	91.43±0.49	91.92^{C}±0.34	0.962	0.001	0.001	0.001
T_1	92.82±0.49	84.47±1.34	88.65^{B}±1.43				
T_2	92.96±0.32	78.62±1.07	85.79^{A}±2.23				
Mean	92.73^{Y}±0.23	84.84^{X}±1.39					

†Sow in control group $T_{0,}$ no supplementary betaine, where as diet of sow in group T_1 and T_2were supplemented with betaine @3g /kg DM either late gestation or whole gestation, respectively; $^{A,B,\ C/X,Y}$Means with different superscripts in a column/ row differ significantly

the studies by Albuquerque *et al.* (2017) and Yang *et al.* (2009). Zou *et al.* (1998) found that betaine enhanced lipase activity and decreased the concentration of triacylglycerols and cholesterol in serum of laying hens. Similarly, Rao *et al*. (2011) conducted an experiment in broiler with betaine supplementation (0 and 800 mg/kg) to diets containing five concentrations (15, 18, 20, 22 and 24 % crude protein) of methionine in a 2×5 factorial study for serum biochemical. Supplementation of betaine at lower levels of met (15 and 18 g/kg CP) significantly increased the concentration of total protein, triglycerides and total cholesterol in serum. In ducks, supplementing betaine at different levels (0, 0.5, 1.0 and 1.5 g/kg diet) resulted in decreased serum concentration of total and LDL-cholesterol with a concomitant increase in HDL-cholesterol (Awad *et al.,* 2014). Betaine may improve the availability of choline, thus providing more choline for the synthesis of very low-density lipoprotein. Thus, the production of very low-density lipoprotein would prevent the deposition of fat in the liver and stimulate the removal of fat from the liver (Yao and Vance, 1989), it also helped to regulate hepatic cholesterol metabolism in chicks (Yun *et al.* 2015). However, in contrast to the results of present study, Fernandez *et al.* (2009) reported that oral supplementation of betaine (4 g/kg) for 15 days before parturition up to lactation in goats resulted in no significance difference in concentration of glucose, albumin, total protein and creatinine. It was reported by Matthews *et al.* (1998) that total protein and albumin were affected by betaine, but the effects were dependent on the energy and crude protein level of the diet. Matthews *et al.* (1998) and Overland *et al.* (1999) reported no significant effect of supplementary betaine on serum or plasma cholesterol concentration. However, in later study, Matthews *et al.* (2001) and Martins *et al.* (2010) reported that betaine supplemented pigs presented higher serum cholesterol.

Based on the results of the present study it can be concluded that dietary betaine supplementation @ 3 g/kg throughout the length of gestation was helpful in decreasing cholesterol and triglyceride concentration in sows.

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