Dietary supplementation of betaine promotes lipolysis by regulating fatty acid metabolism in geese

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ABSTRACT Supplementation of betaine in the diet appears to regulate fatty acid metabolism and decrease fat deposition. This study aims to identify the effects of dietary supplementation of betaine on zootechnical performance, fatty acid synthesis, abdominal fat deposition, and morphology. Three hundred healthy, male, one-day-old Jiangnan White geese of similar body weight were randomly divided into 5 groups, with 6 replicates per treatment and 10 geese per replicate, and given the following amounts of supplementary betaine: 0 (group A), 600 mg/kg (group B), 1,200 mg/kg (group C), 1,800 mg/kg (group D), or 2,400 mg/kg (group E). Feed intake (**FI**), body weight (**BW**), abdominal fat and sebum thickness, clinical blood parameters, hepatic enzyme activity, and abdominal fat morphology were monitored during the experiment. All geese had free access to feed and water throughout the study. Our results indicate that supplementation of betaine increased zootechnical performance at 21 and 42 d of age. The percentage of abdominal fat and sebum thickness of geese at 63 d of age decreased linearly with the addition of betaine ($P < 0.05$). The triglyceride (**TG**) and total cholesterol (**TCHOL**) content of serum decreased with the increased level of betaine when measured at 63 d of age ($P<0.05$). Hormone sensitive lipase (**HSL**) increased with the level of betaine ($P<0.05$). However, dietary betaine appeared to decrease the activity of fatty acid synthase (**FAS**) in the geese at 42 d and 63 d of age ($P<0.05$). The percentage of total area of lipid droplet decreased with the increased level of betaine supplementation. In conclusion, dietary supplementation of betaine increased lipolysis and decreased fat deposition in the finishing period of geese via reducing feed intake. However, the precise mode-of−action is yet unclear and warrants further research.

Key words: betaine, lipolysis, geese, fatty acid metabolism

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INTRODUCTION

Betaine (N, N, N-trimethylglycine), a nontoxic amino acid derivative, has been found in several plants and organisms and donates the methyl group for the synthesis of many substances such as methionine, carnitine, and creatine (Kidd et al., 1997). Recently, it has attracted a lot of attention as an animal nutrition supplement. Betaine not only has physiological functions of improving weight gain, feed efficiency, and muscle yield in birds (Amerah and Ravindran, 2015; Chen et al., 2018), but also plays a vital role in the process of fat metabolism (He et al., 2015; Liu et al., 2019). Moreover, betaine can act as a methyl donor providing its methyl synthesis of lecithin, which facilitates the transport of fat through the body. In addition, betaine donates its libile methyl group, which can be used in transmethylation reactions to synthesize substances such as carnitine and creatine, thus affecting the metabolism of fat in animals. Increased concentrations of carnitine in the liver can promote fatty acid oxidation and reduce the amount of long-chain fatty acids stored in adipose tissue (Fernández-Fígares et al., 2008).

Although, betaine has long been thought to play a role in fat distribution, and to act as a methyl donor. Research of betaine in poultry mainly focuses on chickens, but less on geese. Several studies indicate that betaine might promote fat metabolism through some key enzyme activities. In our previous study, betaine reversed the decreased serum triglyceride and hepatic crude fat to increase slaughter performance (Yang et al., 2017a). Xing et al. (2011) reported that broiler chickens that fed with supplements of 1,000 mg/kg betaine saw significantly decreased mRNA expression of fatty acid

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synthase (**FAS**), lipoprotein lipase (**LPL**), and adipose fatty acid binding protein (**A-FABP**) in abdominal adipose. Moreover, betaine decreased the percentage of CpG methylation pattern. Huang et al. (2008) suggested that the reduction in adipose tissue in finishing pigs might result from decreased acetyl-CoA carboxylase and FAS activity.

Most data in chickens proved that dietary betaine supplementation could regulate fatty acid metabolism and decrease fat deposition. However, lack of information remains an obstacle to understanding the mechanisms by which betaine promotes lipolysis. Furthermore, few information has been published about the effect of betaine on geese. Accordingly, the objective of this study was to investigate the effect of dietary betaine supplementation on zootechnical performance, carcass trait, clinical blood parameters, hepatic enzyme activity, and abdominal fat morphology. Our study on the effect of betaine on lipid metabolism can fill the gap in geese and was of great significance to the development of geese production.

MATERIALS AND METHODS

Ethics Statement

This animal study was approved by the Institutional Animal Care and Use Committee (**IACUC**) of the Yangzhou University Animal Experiments Ethics Committee under permit number SYXK (Su) IACUC 2020-0021. All experimental procedures involving geese were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of the People's Republic of China.

Animals, Experimental Design, and Diets

The study was undertaken using the Jiangnan white goose, a three-line-cross commercial white goose with the characteristics of rapid early growth, intermediate size, superior meat quality, and a strong tolerance and adaptability to coarse feed. A total of 300 healthy male one-day-old Jiangnan white geese were obtained from a commercial hatchery (Changzhou Four Seasons Poultry Industry Co. Ltd., Jintan, China). Birds of similar body weights were randomly divided into 5 groups containing 6 replicates per treatment and 10 geese per replicate. Random sequence was created through GenStat 18 statistical software package (IACR Rothamstead, Hertfordshire, UK) and was divided into 5 diets and using random block sizes of 5. All birds with similar BW were randomly distributed in different pens. The control group (group A) was fed a corn-soybean meal diet, and the 4 experimental diets included betaine at concentrations of 600 mg/kg (group B), 1,200 mg/kg (group C), 1,800 mg/kg (group D), or 2,400 mg/kg (group E). SMI-LYCINE betaine anhydrous 75% was added on top of the basal diet, provided by Beijing Xin Dayang Co. Ltd., (Beijing, China). The composition of betaine is as follows: 75% betaine and the remaining 25% sodium chloride. The basal corn-soybean meal diet was formulated mainly according to NRC (1994) for geese and prior research from our laboratory (Shi et al. 2007; Wang et al. 2010; Table 1). The geese were fed in separate plastic-floored pens with 2 cm^2 square holes that were laid 70 cm above the ground. Feces under the net bed were cleaned daily with an automatic fecal belt. The goose house was cleaned at the end of the trial. The study lasted for 63 d. All geese had access to feed and water *ad libitum* throughout the trial. Water was provided in a half-open plastic cylindrical water tank, and the feed was provided in feeders on one side of each pen. The geese were reared indoors under similar environmental conditions (temperature: 26.0°C ± 3.0°C; relative humidity (**RH**): 65.5 ± 5.0%; lighting period: 16 h; space allocation: 0.5 m^2/gander).

Table 1. Ingredients and nutrient composition of the experimental diets.

Item	1–21 d	22–42 d	43–63 d
Ingredients (%)			
Maize	58.0	59.3	59.5
Soybean meal	30.2	25.1	23.7
Wheat bran	4.8	4.4	4.7
Rice husk	3.4	7.6	8.5
Limestone	0.8	0.8	0.7
Calcium hydrogen phosphate	1.4	1.4	1.5
DL-methionine	0.1	0.1	0.1
Salt	0.3	0.3	0.3
Premix	1.0[1]	1.0[2]	1.0[2]
Total	100.0	100.0	100.0
Calculated values (as-fed basis)			
ME (MJ/kg)[3]	11.32	11.12	11.06
Methionine (%)	0.38	0.35	0.34
Lysine (%)	0.97	0.83	0.80
Nonphytase phosphorus (%)	0.46	0.45	0.45
Analyzed values (as-fed basis)			
Crude protein (%)	19.04	17.02	16.51
Crude fiber (%)	4.54	6.08	6.42
Crude fat (%)	3.11	3.00	2.96
Calcium (%)	0.82	0.80	0.76
Total phosphorus (%)	0.71	0.68	0.67
Betaine (%)	0.029	0.040	0.044

[1]Premix was provided by the Yangzhou University Feed Company (Yangzhou, China). One kilogram of premix contained the following: retinol, 1,200,000 IU; cholecalciferol, 400,000 IU; α-tocopherol, 1,800 IU; 2-methyl-1,4-naphthoquinone, 150 mg; thiamin, 90 mg; riboflavin, 800 mg; pyridoxine, 320 mg; cobalamin, 1 mg; nicotinic acid, 4.5 g; pantothenic acid, 1,100 mg; folic acid, 65 mg; biotin, 5 mg; choline, 45 mg; Fe (as ferrous sulfate), 6 g; Cu (as copper sulfate), 1 g; Mn (as manganese sulfate), 9.5 g; Zn (as zinc sulfate), 9 g; I (as potassium iodide), 50 mg; Se (as sodium selenite), 30 mg.

[2]One kilogram of premix contained the following: retinol, 1200,000 IU; cholecalciferol, 400,000 IU; α-tocopherol, 1,800 IU; 2-methyl-1,4-naphthoquinone, 150 mg; thiamin, 60 mg; riboflavin, 600 mg; pyridoxine, 200 mg; cobalamin, 1 mg; nicotinic acid, 3 g; pantothenic acid, 900 mg; folic acid, 50 mg; biotin, 4 mg; choline, 35 mg; Fe (as ferrous sulfate), 6 g; Cu (as copper sulfate), 1 g; Mn (as manganese sulfate), 9.5 g; Zn (as zinc sulfate), 9 g; I (as potassium iodide), 50 mg; Se (as sodium selenite), 30 mg.

[3]The values were calculated from the ingredients' apparent metabolizable energy (AME) values for chickens.

Sample Collection and Preparation

On d 1, 21, 42 and 63, all birds were weighed to calculate body weight (**BW**), average daily gain (**ADG**). Feed intake (**FI**) by pen on a daily basis to determine the average daily feed intake (**ADFI**). Feed conversion ratio (**FCR**) was calculated from 1 to 21 d, 22 to 42 d, and from 43 to 63 d, and mortality was recorded as it occurred.

On d 21, 42 and 63, 2 geese from each pen were randomly selected for blood sample collection. The butterfly needle with luer adapter was inserted in wing veins of the geese, and 3 mL blood was collected into a disposable negative pressure blood collection vessel. Blood samples were centrifuged for 10 min at 4,500 rpm to obtain plasma for the measurement of biochemical indices. The plasma was stored at −20°C for the analysis of clinical blood parameters.

After taking blood, the geese were killed by cervical dislocation to obtain liver samples. Liver samples (approximately 20 g) were rapidly collected from the right side of the liver in each gander, and stored at −80°C for analysis of the hepatic enzyme activities.

At 63 d of age, 2 geese from each treatment replicate were randomly selected and slaughtered using exsanguination after feed deprivation for 6 h. The geese were disemboweled and sebum thickness was measured by vernier caliper, with care taken to ensure that the caliper was not forced. In addition, the live body weight, carcass weight, eviscerated carcass weight, breast meat (including pectoralis major and pectoralis minor), leg meat (including thigh and drumstick), abdominal fat were extracted and weighed. Internal abdominal fat were rapidly collected for histological studies.

Clinical Blood Parameters

Plasma concentrations of triglyceride (**TG**), total cholesterol (**TCHOL**), high-density lipoprotein (**HDL**), and low-density lipoprotein (**LDL**) were measured using an automatic biochemical analyzer (UniCel DxC 800 Synchron, Beckman coulter, CA).

Hepatic Enzyme Activities

The enzyme activity of hormone sensitive lipase (**HSL**), fatty acid synthase (FAS) and lipoprotein lipase (**LPL**) in the liver of geese were tested using ELISA kits (No. E201911161120; No. E201911271529; E201911171043) purchased from Shanghai Yubo Biotechnology Co. Ltd., (Shanghai, China). Both the inbatch and interbatch coefficients of variation were less than 10%.

Histological Studies

The abdominal fat was immediately fixed in neutral buffered formalin, embedded in paraffin wax, cut into 5-μm thick sections, and stained with hematoxylin and eosin (H&E) as described by Ji and Kaplowitz (2003). Histological alteration was observed and photographed using an inverted microscope (Nikon, Tokyo, Japan). A microscope equipped with TSView 7 software (Tucsen, China) was used to determine the total area of lipid droplets.

Statistical Analysis

The observation unit was the floor pen for zootechnical performance, while for the other variables the observation unit was the individual goose. The experimental data were preliminarily sorted using Excel 2020, and then analyzed with SPSS 20.0 software (Ver. 20.0 for Windows, SPSS, Inc., Chicago, IL). All the data were analyzed by randomized block ANOVA and one-way analysis of variance, linear, and quadratic analysis were performed. Significant differences between the treatments were determined at $P < 0.05$ using Tukey test. The data analysis results were expressed as mean values and standard errors.

RESULTS

Zootechnical Performance

Mortality data were transformed before analysis. Mortality was low (<1%), and there were no treatment effects. As shown in Table 2, BW increased linearly with the rise of betaine at 21 and 42 d of age (P<0.05). However, the BW for groups A and B was significantly higher than that of groups of C, D, and E at 63 d of age (P<0.05). Dietary betaine had no significant effect on ADFI, ADG and FCR from 1 to 21 and from 22 to 42 d of age (P<0.05). ADFI and ADG decreased with the increase of betaine, however, FCR increased linearly with the rise of betaine at 43 to 63 d of age (P<0.05).

Carcass Trait

The effect of different dietary levels of betaine on carcass traits of geese at 63 d of age was shown in Table 3. There was a linear decrease in the percentage of abdominal fat and sebum thickness of geese at 63 d of age with the addition of betaine ($P < 0.05$), and the linear model was Y = 0.783-0.057X and Y=1.502-0.045X, respectively. There was no significant effect of dietary betaine on dressing percentage, percentage of eviscerated yield, percentage of breast muscle, percentage of leg muscle.

Clinical Blood Parameters

The effect of different dietary levels of betaine on serum biochemical indices of geese was shown in Table 4. The TG and TCHOL content of serum were significantly affected by different levels of betaine ($P < 0.05$), decreasing with the increase in betaine when measured at 63 d of age (P<0.05).

Hepatic Enzyme Activities

The effect of different dietary levels of betaine on hepatic enzyme activity of geese was shown in Table 5. Dietary supplementation of betaine had a linear effect

Table 2. Effect of different dietary levels of betaine on the zootechnical performance from one to 63 d of age.

Item[1,2]	Groups[3] A	B	C	D	E	SEM	P-value[a,b] Bet	Linear	Quadratic
BW 1 d (g)	85.42	85.49	85.49	85.56	85.58	0.060	0.932	0.389	0.980
BW 21 d (g)	893.14	932.80	935.68	946.54	970.74	10.293	0.200	0.023	0.784
BW 42 d (g)	2451.74[b]	2583.13[a]	2609.64[a]	2663.45[a]	2688.35[a]	23.748	0.015	0.001	0.281
BW 63 d (g)	3930.88[a]	3918.07[a]	3728.34[b]	3698.98[b]	3663.74[b]	31.475	0.004	0.488	0.340
ADG 1−21 d (g/d)	38.46	40.35	40.49	41.01	42.16	0.490	0.200	0.023	0.784
ADG 22−42 d (g/d)	78.32	79.62	78.07	81.76	81.79	1.031	0.684	0.238	0.763
ADG 43−63 d (g/d)	66.33[a]	62.53[ab]	54.92[bc]	49.31[c]	48.92[c]	0.466	0.006	0.001	0283
ADG 1−63 d (g/d)	66.33[a]	62.53[ab]	54.92[bc]	49.31[c]	58.92[c]	1.636	<0.001	<0.001	0.334
ADFI 1−21 d (g/d)	74.75	77.46	77.44	79.59	80.45	1.018	0.456	0.073	0.860
ADFI 22−42 d (g/d)	226.01	224.57	236.69	232.17	235.77	2.188	0.283	0.085	0.717
ADFI 43−63 d (g/d)	290.76[a]	287.57[a]	270.62[b]	264.52[b]	261.05[b]	3.529	0.009	0.001	0.679
ADFI 1−63 d (g/d)	197.17	196.54	194.91	192.09	192.42	1.202	0.590	0.120	0.283
FCR 1−21 d	1.94	1.92	1.91	1.94	1.91	0.009	0.611	0.496	0.831
FCR 22−42 d	2.90	2.82	3.05	2.85	2.89	0.035	0.299	0.982	0.510
FCR 43−63 d	4.40[c]	4.63[bc]	4.97[ab]	5.41[a]	5.30[a]	0.103	0.002	<0.001	0.395
FCR 1−63 d	3.23[b]	3.23[b]	3.37[a]	3.35[a]	3.34[ab]	0.019	0.028	0.009	0.213

[1]Abbreviation: FCR, feed conversion ratio.
[2]For zootechnical performance results are expressed as means, with n = 6 per treatment.
[3]Group A received only the basal diet; group B received the basal diet supplemented with an additional 600 mg/kg betaine; group C received the basal diet supplemented with an additional 1,200 mg/kg betaine; group D received the basal diet supplemented with an additional 1,800 mg/kg betaine; group E received the basal diet supplemented with an additional 2,400 mg/kg betaine.
[a,b]In the same row, values with different small letter superscripts indicate a significant difference (P<0.05), while those with the same or no letter superscripts indicate no significant difference (P>0.05).

Table 3. Effect of different dietary levels of betaine on carcass traits of geese at 63 d of age.

Item[1]	Groups[2] A	B	C	D	E	SEM	P-value[a,b] Bet	Linear	Quadratic
Dressing percentage (%)	86.69	87.66	86.94	87.27	88.33	0.234	0.190	0.079	0.526
Percentage of eviscerated yield (%)	70.84	71.65	71.23	71.58	72.96	0.239	0.058	0.013	0.328
Percentage of breast muscle (%)	7.78	7.29	7.41	7.82	7.37	0.120	0.513	0.730	0.706
Percentage of leg muscle (%)	13.41	13.90	13.60	14.03	13.08	0.194	0.569	0.703	0.196
Percentage of abdominal fat (%)	3.90[a]	3.94[a]	3.26[b]	3.25[b]	2.89[b]	0.094	<0.001	<0.001	0.875
Sebum thickness (cm)	0.76[a]	0.64[ab]	0.60[ab]	0.56[b]	0.50[b]	0.026	0.029	0.002	0.532

[1]Results are expressed as means, with n = 12 per treatment.
[2]Group A received only the basal diet; group B received the basal diet supplemented with an additional 600 mg/kg betaine; group C received the basal diet supplemented with an additional 1,200 mg/kg betaine; group D received the basal diet supplemented with an additional 1,800 mg/kg betaine; group E received the basal diet supplemented with an additional 2,400 mg/kg betaine.
[a,b]In the same row, values with different small letter superscripts indicate a significant difference (P<0.05), while those with the same or no letter superscripts indicate no significant difference (P>0.05).

Table 4. Effect of different dietary levels of betaine on serum biochemical indices (mmol/L) of geese.

Age	Items[1,2]	Groups[3] A	B	C	D	E	SEM	P-value[a,b] Bet	Linear	Quadratic
21 d	TG	0.52	0.45	0.59	0.67	0.50	0.040	0.428	0.489	0.457
	TCHOL	4.38	4.35	3.94	4.47	4.25	0.094	0.508	0.822	0.484
	HDL	1.49	1.52	1.66	1.50	1.69	0.048	0.538	0.259	0.931
	LDL	1.48	1.37	1.47	1.44	1.52	0.052	0.925	0.699	0.598
42 d	TG	0.45	0.41	0.37	0.32	0.33	0.026	0.477	0.082	0.956
	TCHOL	4.87	4.60	4.55	4.37	4.19	0.117	0.446	0.065	0.974
	HDL	2.10	2.13	2.03	2.28	2.23	0.050	0.562	0.629	0.477
	LDL	1.84	1.64	1.61	1.59	1.70	0.057	0.648	0.417	0.199
63 d	TG	0.985[a]	0.760[b]	0.772[b]	0.668[b]	0.036	0.011	0.011	0.002	0.208
	TCHOL	4.62[a]	4.38[ab]	4.37[ab]	4.00[b]	3.93[b]	0.085	0.046	0.003	0.979
	HDL	2.34	2.48	2.25	2.67	2.67	0.071	0.213	0.080	0.539
	LDL	1.21	1.21	1.10	1.31	1.27	0.037	0.495	0.367	0.420

[1]Abbreviations: HDL, high-density lipoprotein; and LDL, low-density lipoprotein; TCHOL, total cholesterol; TG, triglyceride.
[2]Results are expressed as means, with n = 12 per treatment.
[3]Group A received only the basal diet; group B received the basal diet supplemented with an additional 600 mg/kg betaine; group C received the basal diet supplemented with an additional 1,200 mg/kg betaine; group D received the basal diet supplemented with an additional 1,800 mg/kg betaine; group E received the basal diet supplemented with an additional 2,400 mg/kg betaine.
[a,b]In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while those with the same or no letter superscripts indicate no significant difference (P>0.05).

Table 5. Effect of different dietary levels of betaine on the hepatic enzyme activities of geese.

Age	Items[1,2]	Groups[3]					SEM	P-value[a,b]		
		A	B	C	D	E		Bet	Linear	Quadratic
21 d	HSL	196.40	178.88	179.68	198.79	199.65	4.546	0.391	0.417	0.329
	FAS	573.73	591.23	587.02	578.81	567.67	8.729	0.928	0.711	0.868
	LPL	124.41	142.87	142.46	142.19	137.84	3.949	0.594	0.367	0.196
42 d	HSL	172.51	195.01	204.24	203.77	206.14	4.976	0.172	0.032	0.615
	FAS	629.38	614.64	606.27	589.83	574.72	8.708	0.321	0.036	0.997
	LPL	145.28	147.07	149.75	144.27	141.92	3.521	0.973	0.947	0.956
63 d	HSL	196.99[c]	205.09[bc]	222.43[abc]	226.75[ab]	238.22[a]	4.727	0.027	0.001	0.857
	FAS	623.09[a]	591.80[ab]	583.42[ab]	571.66[b]	555.71[b]	7.278	0.034	0.002	0.881
	LPL	149.41	146.49	143.56	138.88	133.89	3.016	0.528	0.086	0.996

[1]Abbreviations: FAS, fatty acid synthase; HSL, hormone sensitive lipase; LPL, lipoprotein lipase.
[2]Results are expressed as means, with n = 12 per treatment.
[3]Group A received only the basal diet; group B received the basal diet supplemented with an additional 600 mg/kg betaine; group C received the basal diet supplemented with an additional 1,200 mg/kg betaine; group D received the basal diet supplemented with an additional 1,800 mg/kg betaine; group E received the basal diet supplemented with an additional 2,400 mg/kg betaine.
[a,b]In the same row, values with different small letter superscripts indicate a significant difference (P<0.05), while those with the same or no letter superscripts indicate no significant difference (P>0.05).

on the HSL activity of the liver when measured at 42 d and 63 d of age (P<0.05), with HSL increasing with the level of betaine (P<0.05). However, dietary betaine decreased the activity of FAS at 42 d and 63 d of age (P<0.05). There was no significant observed effect of dietary betaine on LPL.

Abdominal Fat Morphology

The effect of different dietary levels of betaine on abdominal fat morphology was shown in Figure 1. Dietary supplementation of betaine had significant effects on the abdominal fat morphology of the geese at 63 d of

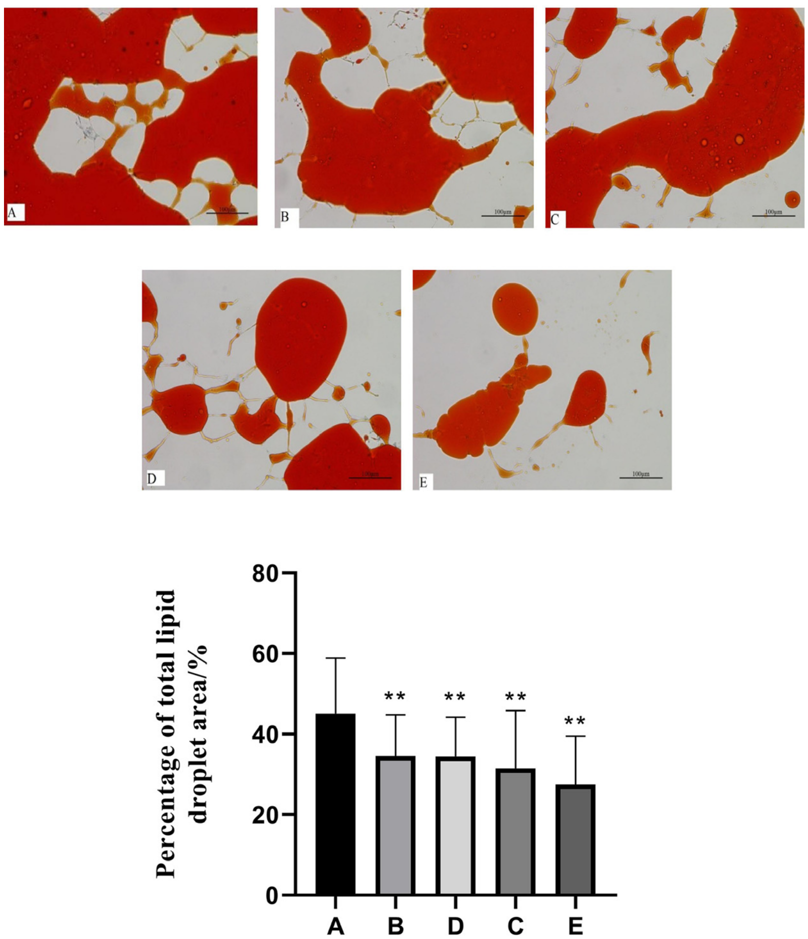

Figure 1. Effect of different dietary levels of betaine on abdominal fat morphology of the geese at 63 d of age. Columns of percentage of total lipid droplet area with ** indicate a significant difference (P<0.05). Results are expressed as means, with n = 12 per treatment. Group A received only the basal diet; group B received the basal diet supplemented with an additional 600 mg/kg betaine; group C received the basal diet supplemented with an additional 1,200 mg/kg betaine; group D received the basal diet supplemented with an additional 1,800 mg/kg betaine; group E received the basal diet supplemented with an additional 2,400 mg/kg betaine. Scale bar−100 μm.

age (P<0.05). The percentage of total area of lipid droplet in group B, group C, group D, and group E were significantly lower than that of group A (P<0.05).

DISCUSSION

Numerous studies on the application effect of betaine on animals have accelerated the development and promotion of betaine as a feed additive; however, the suitable amount at different ages and the mechanism of action are still controversial. The present study demonstrated that supplementation of betaine significantly increased the BW of geese at 42 d of age during the initial period. The geese whose feed was supplemented with 600 mg/kg, 1,200 mg/kg, 1,800 mg/kg, and 2,400 mg/kg betaine had greater BW at 42 d of age compared with the control group, which implied that diets supplemented with betaine may improve the zootechnical performance of birds. The results of our study are consistent with the known effects of betaine on zootechnical performance reported by Hassan et al. (2005) and Chen et al. (2018). Moreover, Rao et al. (2011) also found that BWG of broilers during 1 to 42 d was significantly improved by betaine supplementation (800 mg/kg). However, BW saw a slight but not significant decrease at 63 d of age with the supplementation of betaine during the later growing period. Meanwhile, ADFI was significantly reduced with the addition of betaine during the 43 to 63 d period. With the decrease of feed intake and energy intake, it is logical that fatty acid synthesis and abdominal fat deposition decrease. While energy demands are not met, it is not controversial to say that lipolysis would increase to help meet those demands. So, it is plausible that the mechanism by which betaine increase lipolysis and decrease fat deposition by reducing feed intake. It was also assumed that betaine is increasing lipolysis and, therefore, reduces energy requirements.

The supplementation of betaine finally resulted in improved FCR of 43 to 63 d of age and 1 to 64 d of age. Our previous study (Yang et al., 2017a) also observed a slightly but not significant increase in F/G with the addition of 600 mg/kg betaine. In contrast to our findings, Chand et al. (2017) found that dietary supplementation of 1.5% and 2% betaine increased feed intake and BWG of fast-growing broilers exposed to heat stress but, simultaneously, decreased their FCR. Liu et al. (2019) observed a significant increase in feed intake and BWG of heat-stressed Cobb broilers. A possible explanation for the contradictory results could be that betaine, which was added to geese starting from one-day-old in our experiment, increased lipolysis, which in turn reduced energy requirements and feed intake, and finally resulted in an increase in FCR in the finishing period.

Betaine was effective in improving carcass quality and several published papers have confirmed its effect on fat reduction (He et al., 2015; Dong et al., 2020). Several studies have investigated the mechanisms by which betaine reduces fat deposition. As expected, our data revealed that betaine supplementation significantly decreased the percentage of abdominal fat and sebum thickness of geese at 63 d of age, which was consistent with the result of Chen et al., (2018) that betaine can reduce body fat in broilers. This might be because betaine supplementation increases lipolysis, which in turn reduces abdominal fat. McDevitt et al. (2010) found that betaine supplementation could increase breast meat yield in broilers. Similar results were also reported by Xing et al. (2011) and Dong et al. (2020). However, our results failed to prove that betaine improves muscle growth in geese. It was probably due to the reduced feed intake of geese during the 43 to 63 d period after the supplementation of betaine. The precise mode of action is yet unclear and warrants further research.

The hematological values in the experimental geese were analogous with normal ranges, suggesting adequate and healthy nutrition. Yang et al. (2017a) reported that betaine ingestion of 600 mg/kg resulted in a decrease in plasma TG in geese at 70 d of age. Zhan et al. (2006) also found that dietary betaine supplementation decreased the content of uric acid and TG in the serum of broilers. The results of the present study also showed that supplementation significantly decreased the concentration of TG and TCHOL in serum, which was similar to the results reported by Chen et al. (2018,'2020) as well as Zhang et al. (2019). Their results implied that the molecular mechanism was that betaine inhibited fatty acid synthesis, but, simultaneously, stimulated fatty acid oxidation and lipid secretion in HepG2 cells.

The liver is a major organ for lipid metabolism, in addition to its lipolytic effects on adipose tissue. Yang et al. (2017b) also found that betaine was associated with the suppression of TG, free fatty acid, and total cholesterol accumulation in the liver of geese at 70 d of age. As far as the existing data are concerned, the mechanisms underlying the effects of betaine on lipid metabolism are poorly understood. FAS, HSL, and LPL are major regulators of fatty acid synthesis, limiting enzyme lipogenesis, and decomposition (Cholewa et al., 2014). Huang et al. (2009) proved that betaine supplemented with 1,250 mg/kg significantly reduced ACC and FAS activity, and decreased FAS mRNA expression of finishing pigs. The exact mechanism behind the effects of betaine on geese is yet to be elucidated. Zhan et al. (2006) suggested that the addition of betaine (500 mg/kg and 1,000 mg/kg) significantly increased the activity of HSL in abdominal fat of growing broilers. The results of our present study were consistent with these findings. Our study found that betaine significantly decreased the activity of FAS and increased HSL, which suggested that betaine could promote the decomposition of fat and reduce its synthesis. Further research is therefore needed to clarify these responses in geese.

Betaine-supplemented geese demonstrated smaller percentage of total lipid droplet area compared to the control group. The betaine-supplemented group also showed a decreased percentage of abdominal adipose tissue compared with the control group. This conclusion was supported by Huang et al. (2008) and Mendoza et al. (2010) in pigs who also found that betaine supplementation could promote lipid metabolism. The result was consistent with the known lipid-reducing effects of betaine. The decrease of lipid droplet area was consistent with the above conclusion that betaine can reduce the abdominal adipose tissue of geese. It was assumed that the reduced lipid droplet area was associated with the function of increased lipolysis of betaine.

In conclusion, this study demonstrated that dietary supplementation of betaine increased lipolysis and decreased fat deposition in the finishing period of geese via reducing feed intake and energy requirements. The decreased content of TG and TCHOL with the increasing level of betaine in the serum was consistent with the increased activity of HSL and decreased activity of FAS in the liver.

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DISCLOSURES

The authors have no conflicts of interest to declare.

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