



The effects of dietary betaine supplementation on fatty liver performance, serum parameters, histological changes, methylation status and the mRNA expression level of Spot14 α in Landes goose fatty liver

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ABSTRACT

We evaluated the effects of betaine supplementation on liver weight, liver/body weight, serum parameters and morphological changes. Compared with the control and overfed groups, the geese that were fed the betaine diet showed increased liver weight and decreased abdominal adipose tissue weight compared with the overfeeding groups. Betaine treatment also significantly increased ChE, HDL, LDH and ALT levels ($P < 0.01$ or $P < 0.05$). Decreased macrovesicular steatosis and increased microvesicular steatosis were observed in the betaine-treated group, and the lipid was well-distributed in the betaine supplement group. The expression of S14 α mRNA in the livers of the betaine-treated geese was higher than that in the control or the overfed geese. We performed sodium bisulfite sequencing of the individual alleles of this region (between +374 and –8 base pairs relative to the transcription start site), containing 33 CpG dinucleotides. In the overfed group expressing higher S14 α transcripts, the average methylation at the 33 CpG sites was 87.9%. This contrasted with 69.6% in the control group that showed lower expression of the S14 α gene ($P < 0.01$). However, no significant change in methylation in the transcription start site was found between the betaine-treated geese (82.6%) and the overfed geese (87.9%). These results indicate that the DNA methylation pattern in the S14 α gene transcription start site may not be related to the expression of S14 α transcript in response to betaine supplementation.

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1. Introduction

Fatty goose liver (foie gras) is a well-known delicacy with a delicate texture and delicious flavor due, in part, to high levels of unsaturated fatty acids. Consumers worldwide enjoy foie gras, and there is a large international market. China is a principal producer of foie gras, as there are large numbers of geese flocks in production. Even so, little is known about the relationship between the effective production of these animals and the expression of lipogenic genes. A previous report showed the effects of different breeds and different bulk feeds on the development of geese fatty liver (Fournier et al., 1997; Hermier et al., 1994), and a variety of papers exist that describe the imbalances between hepatic lipogenesis and lipid secretion that are involved in the susceptibility of ducks and geese to hepatic steatosis (Fournier et al., 1997; Hermier et al., 1994, 1999, 2003; Davail et al., 2003). Thus, the susceptibility to geese fatty liver was partly due to a genetic effect (Mourout et al., 2000) and its relationship to the imbalance of lipid metabolism and imbalance resistance.

Betaine is formed by the oxidation of choline and is present in most living organisms (Barak et al., 1996). It was initially introduced to the feed industry as a replacement for methionine and choline in poultry and fish diets, where it is presumed to act both as a methyl donor and as an osmoprotectant (Kidd et al., 1997). In addition, betaine enhances the synthesis of methylated compounds including carnitine and phospholipids (Carter et al., 1995; Chiang et al., 1996). Thus, betaine might be integrally involved in lipid metabolism via its role in phosphatidylcholine synthesis and in FA oxidation, because carnitine is required for transport of long-chain FAs into mitochondria, where they are degraded via β -oxidation (Carter et al., 1995). Moreover, betaine has been accepted as a hepatoprotective agent against alcoholic (Barak et al., 1997) and non-alcoholic steatosis (Neuschwander-Tetri, 2001). Therefore, betaine can be used to enhance the resistance to imbalance between lipid synthesis (increased) and secretion (reduced) due to its hepatoprotective effect.

The thyroid hormone-responsive Spot14 (S14) gene, which encodes a small acidic protein, is localized in hepatic nuclei and acts to transduce hormone- and nutrient-related signals to genes involved in lipid metabolism. Spot14 α has been demonstrated to decrease the expression of a cascade of enzymes in the lipogenic pathway in

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Table 1
Oligo-nucleotide primer pairs for lipogenic genes.

Gene symbol	Primer sequence (5'–3')	Anneal. T (°C)	Amplicon size	GenBank accession number
β-actin	F: 5'-ACCACCGTATTGTTATGGACT-3' R: 5'-TTGAAGGTGGTCTCGTGGAT-3'	65	398 bp	M26111
S14α	F: 5'-GAGGAACGTCCTCTGTGACC-3' R: 5'-GAGGCTTTGCATTTTATTCAG-3'	63	314 bp	DQ227766
Bisulfite-1	F: 5'-TTGGGGTTATGGAGTAGTATT-3' R: 5'-TCTACTCCAAATCTAACTATACCTC-3'	54	382 bp	EU710582

hepatocytes transfected with Spot14α antisense oligonucleotides such as ATP-citrate lyase, fatty acid synthase, and malic enzyme (Kinlaw et al., 1995; Brown et al., 1997; Cunningham et al., 1998; Zhu et al., 2005). Meanwhile, the Spot14α gene model has been used to study hepatic gene regulation by carbohydrates and hormones in lipid metabolism (Ota et al., 1997) and in lipogenic tissues.

The goose S14 gene was cloned and found to share a similar gene organization to that of chickens, ducks and mammals (Su et al., in press). However, little is known regarding the transcriptional level of the goose S14α gene in response to overfeeding in Landes goose livers. Meanwhile, the DNA sequence of the S14α gene is characterized by a large CpG island in the region of the transcriptional start site. Moreover, covalent modification of DNA by methylation of cytosine residues in CpGs is a heritable and reversible epigenetic process that is involved in the regulation of a diverse range of biological processes.

As betaine is involved in both the resistance to hepatic imbalances and lipid metabolism, we designed this study to determine the effect of dietary betaine on body and liver parameters, serum parameters, histological changes, and the transcriptional level and methylation status of the thyroid hormone-responsive Spot14 (S14) gene, which is involved in lipid metabolism by transducing hormone- and nutrient-related signals.

2. Materials and methods

2.1. Animals

A total of 18 healthy male Landes geese (*Anser anser*; BW = 4.0 ± 0.01 kg), obtained from Xingyun Jiangsu, were fed a commercial diet to the age of 10 weeks. From 10 to 12 weeks, the feed restriction was progressively released to increase the volume of the digestive tract and to initiate the metabolic adaptation to overfeeding (500 g/d). At 13 weeks (85 d), the geese were divided into three groups (Table 1). Geese in the first group ($n=6$) continued the control diet and were allowed to feed *ad libitum* (150 g/d) on a diet containing 2600 kcal and 138 g/kg protein and up to 500 g/kg grass. The remaining geese ($n=12$) were switched to an overfeeding diet (420 g/d), which consisted of two-thirds salted and boiled maize (3370 kcal/kg, 90 g protein/kg and 4.5 g fat/kg) to which 0.4% waterfowl fat was added and one-third (by volume) of water. Geese fed the overfeeding diet were fed six meals per day for three weeks (with the overfeeding diet without betaine). Of the geese that were fed the overfeeding diet, six were also fed betaine (Genetime Biotech Co., Nanjing, China) as a dietary supplement (1 g/d/goose). At week 15, the geese were slaughtered, and blood samples were collected approximately 24 h prior to slaughter. All individuals were weighed at slaughter, and the liver and abdominal adipose tissue were also weighed after dissection. The animals were cared for and slaughtered according to the practices approved by the Nanjing Agricultural University Animal Ethics Committee.

2.2. Measurement of serum parameters and liver triiodothyronine

Serum was separated by centrifugation at 3000 g for 15 min and stored at -20 °C. The measurements of alanine transaminase (ALT; EC

2.6.1.2) (kinetic method, Daiichi Pharmaceutical Co. Ltd, Japanese), γ-glutamyl transpeptidase (GGT; EC 2.3.2.2) (Szasz method, Shanghai Forum Long March Medical Science Co. Ltd, China), cholinesterase (ChE; EC 3.1.1.8) (P-hydroxyl-benzoic acid choline; Nakamura Dental Mfg Co. Ltd, Japan), triglyceride (TG) (GPO-PAP, Biosino Biotechnology Company Ltd, China), high-density lipoprotein cholesterol (HDL) (Direct Assay Method, Daiichi Pharmaceutical Co. Ltd, Japanese), low-density lipoprotein cholesterol (LDL) (Direct Assay Method, Daiichi Pharmaceutical Co. Ltd, Japanese), and lactate dehydrogenase (LDH; EC 1.1.1.27) (Colorimetric Method, Shanghai DF Biochemical Technology Co. Ltd, China) were determined using an automatic multi-function-biochemical analyzer (Hitachi Ltd, Japan).

2.3. Histological studies

The livers were 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). To analyze liver lipid infiltration, hepatocytes were stained with Sudan Yellow in all three groups of geese ($n=6$ per group). The livers were fixed in neutral 10% formalin for 24 h, rinsed in 70% ethanol and immersed in Herxheimer's solution (5% Sudan IV in ethanol and acetone) at room temperature for 15 min (Del Boccio et al., 1990). After transferring the tissues into 80% ethanol for 20 min and washing in running water, lipid infiltration into the livers was evaluated by calculating the area of stain (Henry and Bentley, 1981).

2.4. RNA isolation and RT-PCR

Total RNA was isolated from tissues using TRIZOL reagent (Invitrogen, USA) according to the manufacturer's instructions, and the quality of the total RNA was determined using a spectrophotometer at 260/280 nm (OD260/OD280 = 1.8–2.0). The integrity of the ribosomal RNA bands was confirmed on agarose gels. Single-strand cDNA synthesis was carried out from 1 μg of total RNA by reverse transcription. After denaturation at 70 °C for 10 min, the RNA samples were incubated in 1× PCR buffer, 0.5 mM deoxynucleoside triphosphate mix, 4 μM oligo (dT) primer, 32 U RNase and 200 U M-MLV reverse transcriptase (Promega) in a final volume of 25 μL. This

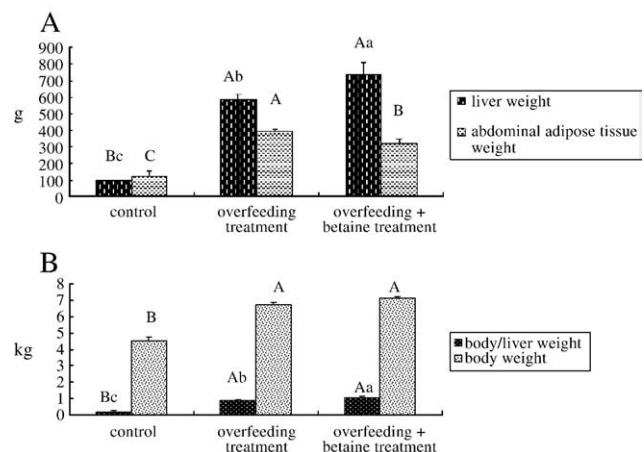


Fig. 1. The effects of overfeeding and betaine supplementation on body weight parameters in geese. (A) Liver mass (g) and abdominal adipose mass (g). (B) Liver/body weight and body mass (kg). Different superscript letters indicate significant differences (capital letters: $P < 0.01$; lower-case letters: $P < 0.05$). Each value represents the mean of six observations ± standard error.

Table 2
The effect of betaine supplementation on the serum parameters of Landes geese.

Group	Serum parameters						
	ALT (U/L)	GGT (U/L)	LDH (U/L)	ChE (U/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Control	18.39 ± 1.92 ^C	2.1 ± 0.26	573.67 ± 137.34	0.07 ± 0.18 ^{Bc}	1.49 ± 0.36 ^B	2.63 ± 0.19 ^C	1.01 ± 0.01 ^b
Overfeeding diet	58.42 ± 7.35 ^B	3.65 ± 2.14	1285.67 ± 277.69 ^b	0.61 ± 0.15 ^{Bb}	4.99 ± 0.62 ^A	3.92 ± 0.23 ^B	1.22 ± 0.23 ^a
Overfeeding + betaine diet	131.57 ± 24.4 ^A	4.57 ± 1.56	2597.14 ± 325.57 ^a	2.56 ± 0.17 ^{Aa}	4.75 ± 0.53 ^A	6.56 ± 0.59 ^A	1.34 ± 0.12 ^a

Different superscript letters indicate significant differences (capital letters: $P < 0.01$; lower-case letters: $P < 0.05$) within each column. Each value represents the mean of six observations ± standard error.

reaction was maintained at 37 °C for 50 min, and the complementary DNA (cDNA) was stored at –20 °C.

2.5. Real-time quantitative PCR

Primer sequences were designed using the software Primer 5.0 and synthesized by TaKaRa (Table 1). The specificity of the amplification product was further verified by electrophoresis on a 0.8% agarose gel and by cDNA sequencing. The results for each gene are presented as ratios relative to β -actin to correct for differences in the amounts of template cDNA used.

Real-time quantitative PCR was carried out in a final volume of 20 μ L containing 1 μ L RT, 1 U EX Taq HS DNA polymerase (TAKARA, Japan), 4 μ L 5 \times PCR Buffer (100 mM Tris–HCl pH 8.3, 500 mM KCl, 0.3 mM dNTPs, 3.75 mM MgCl₂, and 0.5 Mm each primer. The PCR cycling program was as follows: initial denaturation for 1 min at 95 °C, followed by 40 cycles of 95 °C for 10 s, annealing for 15 s (temperature in Table 1), 72 °C for 15 s, plate-reading every other 0.2 °C from 65 °C to 94 °C for drawing melting curves, and a final extension step of 72 °C for 5 min.

A standard curve was generated by amplifying serial dilutions of known quantities of cDNA fragments prepared from the cloned genes, and the dynamic range of the standard curves spanned four orders of magnitude. The efficiency of the PCR reaction was determined for each primer pair by determining the slope of the standard curves obtained from the serial dilution analysis of the cDNA to ensure that the efficiency ranged from 95 to 100%. Fluorescent data were analyzed with the iCycleriQ Optical System Software Ver.3.0a (Bio-Rad) and

converted to cycle threshold values. The relative mRNA concentrations of these genes were determined by transformation using common logarithms and are represented in arbitrary units.

2.6. Sodium bisulfite treatment and methylation-specific polymerase chain reaction (MSP)

Genomic DNA was isolated from hepatic tissues using the standard method: treatment with SDS and Proteinase K followed by phenol/phenol:chloroform:isoamyl alcohol/chloroform:isoamyl alcohol extraction and ethanol precipitation in the presence of salt (Sachan and Raman, 2006). The DNA concentration was estimated by UV spectrometry at 260–280 nm. Approximately 1 μ g of DNA was modified with sodium bisulfite and subjected to MSP as described previously (Herman et al., 1996). The reactions were hot-started at 95 °C for 5 min and held at 80 °C before the addition of 0.625 U of Taq polymerase (Sigma-Aldrich, St. Louis, MO, USA). The temperature conditions for thermocycling were as follows: 35 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s, followed by 1 cycle of 72 °C for 5 min. The PCR products were separated on 2.5% agarose gels and visualized by ethidium bromide staining.

2.7. Sodium bisulfite DNA sequencing

The 382-bp PCR products contained 33 CpG sites located in S14 α the first exon region near the transcription start site. Reactions were hot-started at 95 °C for 5 min and held at 80 °C before the addition of 1.25 U of Taq polymerase (Sigma). The temperature

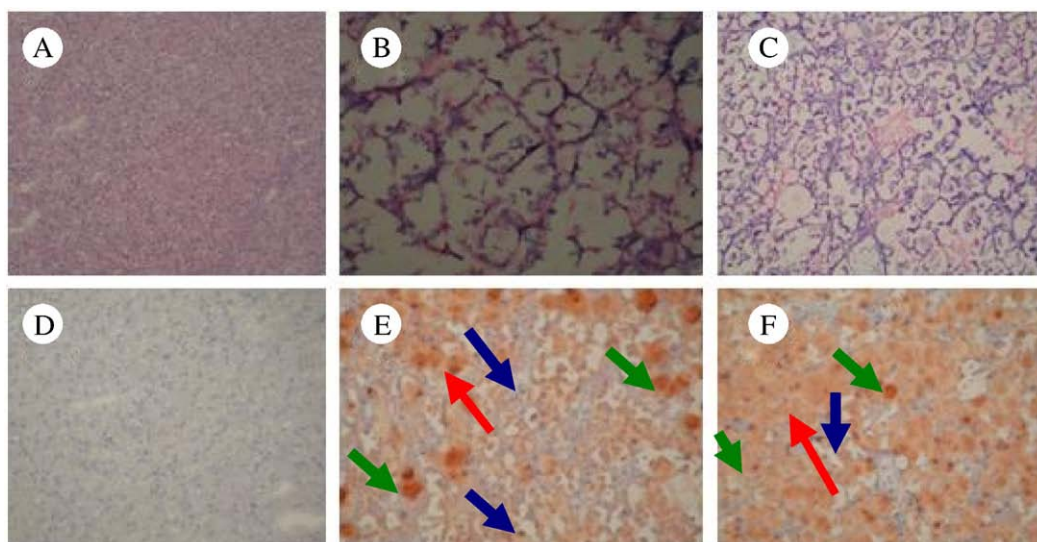


Fig. 2. Liver histology (200 \times). A–C: H&E-stained liver tissue. D–F: Fat-stained liver tissue. A, D: control group; B, E: positive group (the overfeeding diet without betaine supplementation); C, F: the betaine-treated group (the overfeeding diet + betaine). Livers from geese that were fed the control diet showed regularly shaped hepatocytes with large nuclei and some lipid vacuoles in the cytoplasm (these did not affect the hepatocyte size). Livers from geese that were fed the overfeeding diet exhibited swollen hepatocytes with numerous lipid vacuoles of varying size in their cytoplasm. Lipid deposition (yellow color) was increased in the positive control group (E), with marked steatosis and vacuolus change (blue arrow) compared with the negative control group (D). Diffuse yellow lesions and swollen hepatocytes were observed in the betaine-treated group (F) with decreased macrovesicular steatosis (green arrow) and increased microvesicular steatosis. Therefore, the lipid was well-distributed in the betaine-supplementation group (red arrow).

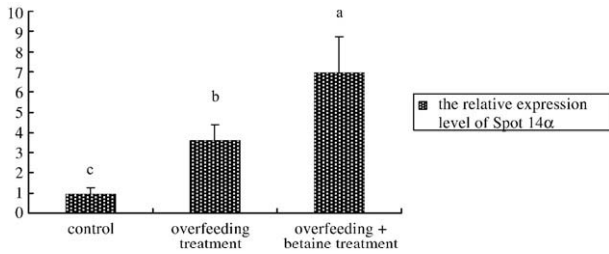


Fig. 3. Real time PCR results of the relative abundance of mRNA transcripts for Spot14 α in response to betaine treatment. The small letter represents a significant difference ($P < 0.05$). Each value represents the mean of six observations \pm standard error.

conditions for the PCR were as follows: 35 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. The PCR products were purified using a commercially available kit (Qiagen, Hilden, Germany), cloned into the TA vector pCR2.1 TOPO (Invitrogen, Carlsbad, CA, USA) and transformed into *E. coli*. Plasmid DNA from isolated clones containing the insert was purified and finally subjected to cycle sequencing using fluorescent Big Dye technology (ABI automated sequencing).

2.8. Statistical analysis

The data are shown as means \pm standard error (SE) for each treatment. All data were tested by ANOVA (general linear model – One-way analysis of variances) using SPSS13.0 software. The means were compared using Tukey's least significance difference (LSD) test at two significant levels of 0.05 and 0.01.

3. Results

3.1. Body and liver parameters

All geese (except the control group) completely consumed the diet provided. As shown in Fig. 1, betaine treatment led to an increase in liver weight and liver/body weight compared with the control and overfeeding groups ($P < 0.05$). The betaine-treated group also had greater body weight and abdominal adipose weight compared with the control group (both $P < 0.01$), and decreased abdominal adipose tissue weight compared with the overfeeding group ($P < 0.01$). However, no difference in body weight was found between the geese fed the overfeeding diet without or with betaine.

3.2. Determination of the biochemical parameters of Landes goose serum

The results of the serum biochemical assays are shown in Table 2. Compared with the control and overfeeding diets (Table 2), betaine treatment significantly increased ChE, HDL, LDH and ALT levels ($P < 0.01$ or $P < 0.05$). ALT ($P < 0.01$), TG ($P < 0.01$), HDL ($P < 0.01$), LDL ($P < 0.05$), and ChE ($P < 0.05$) were increased with the overfeeding/no betaine diet compared with the control diet. There were no differences in GGT levels between the three groups.

Table 3

The correlation between the expression of lipogenic genes in livers and the serum parameters of Landes geese.

Gene		Liver/body weight	ALT (U/L)	GGT (U/L)	ChE (U/L)	TG (mmol/L)	H-cholesterol (HDL) (mmol/L)	L-cholesterol (LDL) (mmol/L)
Spot14 α	Correlation coefficient ^a	0.74	0.643	-0.061	0.429	0.032	-0.075	-0.273
	P value ^b	0.01	0.005	0.816	0.086	0.902	0.774	0.290

^a The values in Table 3 were due to the correlation of the level of expression with the genes, liver/body weight, and serum parameters.

^b The P value is the significance level.

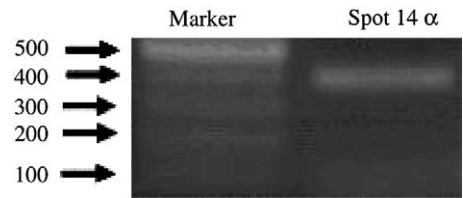


Fig. 4. Representative gel photograph of a PCR-amplified fragment. The fragment (382 bp) was cloned to analyze the methylation status of S14 (Spot 14).

3.3. Morphological changes in response to betaine treatment

Tissue sections from the goose liver were stained with H&E and Sudan IV stains for histological examination (Fig. 2), revealing hepatic lipid deposits appearing as small vacuoles within the cytoplasm of the liver cells in positive (Fig. 2B), but not negative (Fig. 2A) control geese. Betaine-treated geese had smaller lipid droplets than the positive control geese (Fig. 2C). Sudan IV staining was used to identify grossly visible areas of lipid accumulation. We found that lipid deposition (yellow color) was increased (Fig. 2E) in the positive control group, with marked steatosis and vacuolus change (blue arrow) compared with the negative control group (Fig. 2D), but without steatohepatitis (Ishak et al., 1995). Lesions of a diffused yellow nature and swelling of hepatocytes were observed in the betaine-treated group (Fig. 2F) with decreased macrovesicular steatosis (green arrow) and increased microvesicular steatosis. Lipids were therefore well-distributed in the betaine supplementation group (red arrow).

3.4. mRNA expression level of S14 α in Landes goose livers and its relationship with serum biochemical parameters

The real time PCR results for the relative abundance of mRNA transcripts of the S14 α gene are shown in Fig. 3. The expression of this gene in the overfed geese was higher than that in the geese that were fed the control diet. Compared to both the control and overfeeding groups, overfeeding + betaine treatment resulted in a higher expression level of S14 α mRNA in the fatty livers. The expression of the S14 α gene in the liver was correlated with the liver/body weight and the serum ALT concentrations ($P < 0.01$, Table 3).

3.5. Methylation of S14 α gene in goose livers in response to overfeeding

We sequenced individual alleles of this region (between +374 and -8 base pairs relative to the transcription start site), which contains 33 CpG dinucleotides. Based on MSP data, dense methylation throughout the entire region proximal to the S14 α transcription start site was observed, with only rare non-methylation of a few CpG sites (Figs. 4 and 5). In the overfed group expressing higher S14 α transcripts, the average methylation at the 33 CpGs sites was 87.9% in contrast to 69.6% in control group that showed lower expression of the S14 α gene ($P < 0.01$). However, no significant change of methylation in the transcription start site was found between betaine treatment group (82.6%) and overfed geese (87.9%). These results indicate that the DNA methylation pattern at the S14 α gene transcription start site

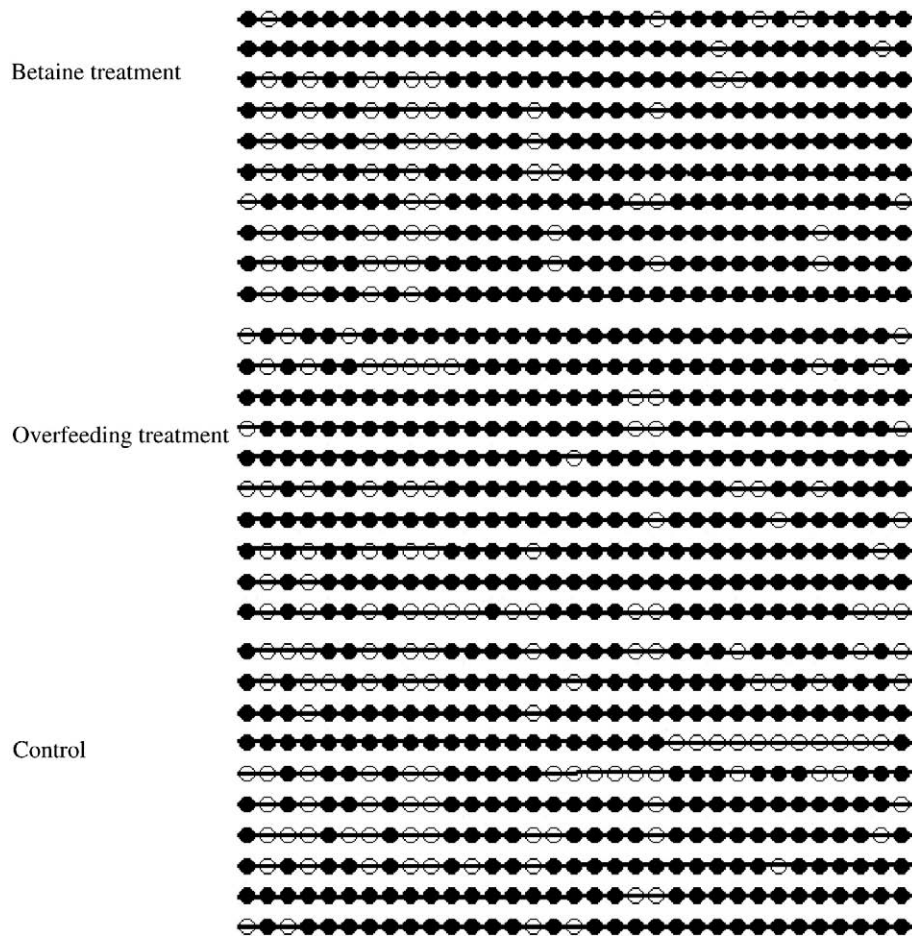


Fig. 5. Spot14 α DNA methylation in Landes geese livers in response to betaine treatment. Bisulfite sequencing results for betaine treatment, overfeeding and control group. Each line represents an individual bacterial clone that was sequenced. Filled circles indicate methylated CpG site; open circles, unmethylated CpG site.

may not be related to expression of the S14 α transcript in response to betaine supplementation.

4. Discussion

Hepatic steatosis of Landes geese results from a dramatic increase in *de novo* lipogenesis induced by overfeeding associated with an imbalance between lipid synthesis (increased) and secretion (reduced). Providing carbohydrate-rich diets for less than 2 weeks (overfeeding) may result in increased liver weights (Hermier et al., 1994) due to *de novo* synthesis and the storage of triglycerides in the liver (Hermier et al., 1991). Similarly, consumption of a high carbohydrate diet can dramatically increase DNL (the *de novo* lipogenic gene) in the human liver (Hudgins et al., 2008), the principal organ responsible for the conversion of excess dietary carbohydrate into triglycerides (Uyeda et al., 2002).

Betaine is a hepatoprotective agent against alcoholic (Barak et al., 1997) and non-alcoholic steatosis (Neuschwander-Tetri, 2001). However, there have been no reports investigating the impact of betaine supplementation on the generation and development of fatty livers in geese. Therefore, in the present study, we have identified several significant differences between the livers of Landes geese fed an overfeeding diet (+) betaine and the livers of geese that were fed an overfeeding diet (–) betaine.

Betaine-treated geese had smaller lipid droplets and attenuated vacuolus change compared to the carbohydrate-only treatment group. However, betaine treatment led to an increase in liver weight ($P < 0.05$) and liver/body weight ($P < 0.05$), compared with the negative control and overfeeding groups. Consequently, it appears that, in our study, betaine-treated geese had more hepatocytes containing smaller lipid

droplets and with less apoptosis. The betaine-treated group also had decreased abdominal adipose weight compared with the positive control group ($P < 0.01$). This result is consistent with the known effects of betaine on fat reduction (Huang et al., 2008; Fernandez et al., 1998).

Fournier et al. (1997) observed that Landes geese are more susceptible to liver steatosis than Poland geese and that they have lower HDL concentrations in the blood. In our study, betaine decreased hepatic steatosis and increased the plasma HDL levels. This suggests that hepatic steatosis in Landes geese may be related to plasma HDL concentrations and that both may be changed by betaine treatment. Furthermore, in Landes geese, the liver weights and triglyceride contents were both negatively correlated with the concentration of plasma phospholipids, especially HDL (Fournier et al., 1997). This may indicate that plasma HDL is involved in the protective effect of betaine. Betaine treatment significantly increased ALT levels ($P < 0.01$ or $P < 0.05$) compared to the positive control group. This result was contrary to the finding that betaine treatment with ethanol caused decreases in plasma ALT in guinea pigs (Balkan et al., 2004). The effect appeared to be highly dependent on the time lapse of treatment (Kim et al., 1998). The level of LDH was measured in order to determine the cytotoxicity of the hepatocytes (Kharbanda et al., 2005). However, no significant change in the concentration of LDH was observed when the apoptosis of the hepatocytes was decreased by the betaine treatment. Therefore, the effects of betaine treatment may not be caused by the levels of LDH. In the present study, it is not surprising that the serum lactate dehydrogenase and ALT concentrations increased after betaine treatment, when steatosis was attenuated.

In fatty livers from Landes geese, a relative deficiency in phospholipid synthesis together with enhanced phospholipid secretion may be

limiting factors of hepatocyte hypertrophy and subsequent steatosis (Fournier et al., 1997). Moreover, acting as a methyl donor (via transmethylation), betaine may enhance the synthesis of methylated compounds, including carnitine and phospholipids (Carter et al., 1995; Chiang et al., 1996), and result in a difference in DNA methylation (Oliva et al., 2009). Therefore, the lipid deposits of the fatty liver may be caused by the methylation of CpG (located in the start site of genes) and the rate of gene transcription in the geese that were treated with betaine.

Here, the expression of S14 α mRNA in the liver of geese that were treated with betaine was higher than that in geese fed either the control or the overfeeding diets ($P < 0.05$). This may imply that in Landes goose liver, excess dietary carbohydrate is converted into (*de novo* synthesized) triglycerides. This was due to the transducing of hormone- and nutrient-related signals by the S14 protein to genes that are involved in lipid metabolism (Campbell et al., 2003). In addition, the protein also acts as a positive or negative cofactor of the thyroid hormone receptor, which is involved in the regulation of malic enzyme gene expression (Chou et al., 2007). Furthermore, enhanced malic enzyme activity may be a feature of lipid synthesis in the more-susceptible Landes geese (Mourot et al., 2000).

Su et al. (2009) identified high GC levels in the (61–327) region of the goose S14 α gene. Meanwhile, the methylation of high GC content-containing sequences may be correlated with gene mRNA expression and the suppression of translation (Kawane et al., 2005; Le and Maizel, 2007). Thus, epigenetic analysis of the S14 α gene would be required to study lipid metabolism in geese. A total of 33 CpG sites were found in the transcription start region of the S14 α gene (–8/+374). During the period of overfeeding, the methylation pattern at the 33 CpG sites was shifted generally from hypomethylation to hypermethylation. This does not accord with the elevated expression of the S14 α gene from the suppression of mRNA expression. This indicates that a repressor may be involved with the CpG island (Vidaković et al., 2009), thereby regulating mRNA expression of the S14 α gene. However, no significant change of methylation was found between the betaine treatment group (82.6%) and the overfed geese (87.9%) at the transcription start site. This finding suggested that the dose of betaine given (as a methyl donor) was not sufficient to methylate the CpG island. However, the increase in the level of S14 α mRNA may be related to transmethylation by the betaine supplement.

5. Conclusions

Compared with the control and overfeeding groups, the geese that were fed the betaine diet had increased liver and decreased abdominal adipose tissue weight. In contrast to the control and overfeeding diets, betaine supplementation significantly increased ChE, HDL, LDH and ALT levels ($P < 0.01$ or $P < 0.05$). In addition, the lipids in the betaine supplement group were well-distributed. The expression of S14 α mRNA in the livers of the betaine treatment group was higher than that in the control or overfed geese. However, no significant change of methylation at the transcription start site was found between the betaine-treated (82.6%) and overfed geese (87.9%). Meanwhile, in the overfed group that expressed the S14 α transcripts at a higher level, the average methylation at the 33 CpGs sites was 87.9%, contrasting with 69.6% in the control group that showed lower expression of the S14 α gene ($P < 0.01$). These results indicate that a repressor may be involved in the CpG island, regulating the level of S14 α gene mRNA expression. Therefore, lipid accumulation and the methylation status and expression levels of both mRNA and the S14 α protein, with respect to carbohydrate and betaine supplementation, require further study in Landes goose hepatocytes.

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