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The Effect of Hydrated Sodium Calcium Aluminosilicate on Fatty Liver and the Composition of the Intestinal Microbiota in Overfed Landes Geese

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■Keywords

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ABSTRACT

Goose fatty liver is a delicious food product and the overfeeding will cause the abnormal physiology of the geese. The objective of this study was to investigate the effect of supplementation with hydrated sodium calcium aluminosilicate (HSCAS) on the fatty liver, ileal and cecal microbiota of Landes geese during overfeeding. Sixty 70-day-old Landes geese (body weight= 3.0 ± 0.05 kg) were randomly divided into three groups, two of which were overfed with whole corn supplemented with or without HSCAS for 20 days when the fatty liver reaches to the maximum size and the negative control group was *ad libitum* access to the corn basal diet. The intestinal contents of the ileum and cecum from three geese per group were used for high-throughput sequencing. As a result of this study, the HSCAS-treatment led to an increase in relative liver weight (p<0.05) of geese compared with the overfeeding control group. The richness and diversity of the bacterial communities decreased in the ileum and ceca after overfeeding. Overfeeding increased the relative abundance of *Firmicutes*, especially *Lactobacillus*, in ileal samples. HSCAS supplementation increased the relative abundance of *Lactobacillus,* and decreased the relative abundance of *Actinobacillus* in the ileum and the relative abundance of *Erysipelotrichi, Bacteroides* and *Escherichia* in the ceca. Bacterial richness indicators were also increased in samples from ileum and ceca after HSCAS supplementation. In conclusion, dietary HSCAS supplementation promoted liver performance in overfed Landes geese. HSCAS treatment had a beneficial effect on the intestinal microbiota composition in geese during the overfeeding.

INTRODUCTION

The goose liver has a high capacity for fat accumulation, and the Landes goose is highly susceptible to hepatic steatosis (Lu *et al.*, 2015). During overfeeding (OF), the birds were fed exclusively with corn, which is rich in carbohydrates (especially starch). This diet induces hepatic steatosis, resulting in storage of fatty acids in the liver and producing foie gras (Davail *et al.*, 2003; Liu *et al.*, 2016). Furthermore, OF has an effect on the bacterial composition of the microbiota in these ducks. This diet has been shown to affect bacterial communities (Vasai *et al.*, 2014a; Vasai *et al.*, 2014b).

The intestinal microbiota has a symbiotic relationship with the host and plays a major role in animal physiology (Vasai *et al.*, 2014b). For example, intestinal bacteria can affect the gut morphology and nutrition and stimulate the immune response to protect against pathogens (Leser & Molbak, 2009). Culture-dependent methods have shown that facultatively anaerobic groups of bacteria are the predominant flora of the small intestine, whereas obligate anaerobes comprise nearly the entire microbial population of the cecum (Torok *et al.*, 2011). A study

of two parental genetic types of ducks (Pekin and Muscovy) showed that the ileal and cecal microbiota were mainly composed of *Firmicutes* and *Bacteroidetes* (Vasai *et al.*, 2014a). Different stresses experienced by animals, such as a change in diet, can modify the composition of the microbiota (Serino *et al.*, 2012).

Mycotoxins can be harmful to hosts. Mycotoxin-contaminated feed may lead to consequences such as poor growth performance and meat quality, low nutrient digestibility, and increased disease incidence due to immunosuppression, all of which may result in economic losses (Swamy *et al.*, 2002; Shang *et al.*, 2016). Aflatoxin (AF) is a mycotoxin that particularly affects poultry and often occurs in naturally contaminated feeds (Aravind *et al.*, 2003; Murugesan *et al.*, 2015). A study with chickens showed that dietary supplementation with hydrated sodium calcium aluminosilicates (HSCAS) was effective in binding AF molecules in the gastrointestinal tract, making them unavailable for adsorption and therefore alleviating aflatoxicosis (Chen *et al.*, 2014). In the poultry industry, the interest in HSCAS is increasing. Because the use of antibiotics in food animals for growth promotion and disease prevention may lead to antibiotic resistance in humans and animals, several countries have restricted the use of antibiotics in livestock feed as a public health measure (Salim *et al.*, 2013). The beneficial effects of dietary HSCAS have been recognized for the last three decades (Tanpong *et al.*, 2017), and it may serve as an alternative to antibiotic growth stimulants without clinical side effects (Gilani *et al.*, 2016). Although their nutritional properties have been widely studied in animals, to date, few reports are available on goose liver changes in response to OF. High-throughput 16S sequencing of major intestinal bacteria may help explain the effects and mechanisms of HSCAS in geese, as no similar studies have been published.

Therefore, the objectives of this study were to determine the effects of an HSCAS supplement in overfed geese by making an inventory of fatty liver and intestinal microbiota in both ileum and ceca, and the effect of overfeeding to know the impact of a food stress on the microbiota was studied using a high-throughput amplicon sequencing.

MATERIAL AND METHODS

Experimental design

All experimental procedures involving geese were conducted in accordance with the guidelines set by the Animal Care and Use Committee of Zhejiang University for the care of animals for research purposes. Geese were raised on a farm belonging to the ChangXing Glory Goose Industry Co. Ltd., Huzhou, China.

A total of 60 healthy 70-day-old Landes geese (BW = 3.0 ± 0.05 kg), hatched on the same day and bred under natural light and temperature conditions. They were randomly divided into three groups: 1) ad libitum access to a corn basal diet containing no additives (negative control group, Control I); 2) overfed with a corn basal diet (positive control group, Control II); 3) overfed with a corn basal diet supplemented with 0.3% HSCAS product (treatment group). Each treatment had four replicate pens with five geese in each pen. The movement of the birds was not restricted before day 0 of overfeeding, but all of the birds were restricted as they were moved to cages for the period of adaptation and OF. A 5-day-long pre-OF period prepared the OF group for formal OF, which lasted 20 d. The OF procedure and diet regimes were implemented as previously described (Lu *et al.*, 2015). Routine husbandry was carried out throughout the experiment.

Tissue sampling

The geese were sacrificed at day 20 of OF. Five birds from each group were randomly selected for weighting, scalding and de-feathering. Livers and the carcass were weighed. The intestinal content from the ileum and cecum was collected, and the samples were stored at –20 °C. The intestinal contents of the ileum and cecum from three geese per group were used for high-throughput sequencing, and analyses were performed by Beijing Novogene Biotechnology Co., Ltd (Xie *et al.*, 2016).

DNA extraction, library preparation, and sequencing of 16S rRNA amplicons

Total DNA from ileal and cecal samples was extracted using the CTAB/SDS method. DNA concentration and purity were monitored on 1% agarose gels. Based on the concentration, the extracted DNA was diluted to 1 ng/μL using sterile water and stored at -20 °C until PCR was performed. Amplicons of the V4 region of the 16S rRNA of samples were amplified using bacterial primers 515F (GTGCCAGCMGCCGCGGTAA, forward) and 806R (GGACTACNNGGGTATCTAAT, reverse) (Caporaso *et al.*, 2010). All PCR reactions were carried out using Phusion High-Fidelity PCR Master Mix (New England Biolabs).

A mix of the same volume of 1× loading buffer (containing SYB green) and PCR products was subjected to electrophoresis on a 2% agarose gel for detection of amplicons. Samples with a bright band between

400–450 bp were chosen for further experiments. PCR products were mixed in equidensity ratios. Then, the mixture PCR products was purified using a Qiagen Gel Extraction Kit (Qiagen, Germany) (Nylund *et al.*, 2010).

Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, USA) according to the manufacturer's recommendations, and index codes were added. Library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina HiSeq2500 platform, and 250-bp paired-end reads were generated (Du *et al.*, 2016).

Paired-end read assembly and quality control

Paired-end reads was assigned to samples based on their unique barcode and truncated by removing the barcodes and primer sequences. Paired-end reads were merged using FLASH (version 1.2.7); the splicing sequences were called raw tags. Quality filtering of the raw tags was performed under specific filtering conditions to obtain high-quality clean tags based on the QIIME (version 1.7.0) quality control process (Bokulich *et al.*, 2013). The tags were compared with a reference database (the GOLD database, http://drive5.com/uchime/uchime_download.html) using a UCHIME algorithm to detect chimeric sequences, and then chimeras were removed (Haas *et al.*, 2011). Finally, effective tags were obtained.

Taxonomic classification and statistical analysis

Sequences analyses were performed using Uparse software (Uparse version 7.0.1001). Sequences with ≥ 97% similarity were assigned to the same operational taxonomic units (OTUs). Representative sequence for each OTU was screened for further annotation. For each representative sequence, the GreenGene Database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) was used with the Ribosomal Database Project (RDP) Classifier (version 2.2) algorithm to annotate taxonomic information. In order to determine the phylogenetic relationships of different OTUs, and the differences between dominant species in different samples (groups), a multiple sequence alignment was generated using MUSCLE (version 3.8.31). OTU abundances were normalized using a standard of sequence numbers corresponding to the sample with the least sequences. A subsequent analysis of alpha and beta diversities was performed based on this normalized data. Alpha diversity indices, including the observed species, Chao1 (the estimated true species diversity of a sample), Shannon (biodiversity), ACE (coverage of sampling), and Good's coverage indices, were used to analyze the complexity of species diversity in a sample All of these indices were calculated in QIIME (version 1.7.0) and displayed using R software (version 2.15.3). A beta diversity analysis was used to evaluate differences in species complexity between samples, and beta diversity based on both weighted and unweighed Unifrac were calculated in QIIME (version 1.7.0). A *t*-test was performed to determine the statistical significance of differences.

Statistical analyses

Carcass, liver weight, and relative liver weight data are presented as mean ± SE. Data were analyzed as a completely randomized design. A comparison of variables was performed using an independent-samples t-test and one-way ANOVA with SPSS v. 20.0 software (SPSS Inc., 2011). Differences among treatment means were determined using Duncan's multiple range test. The results were considered significantly different at $p \leq 0.05$.

RESULTS

Body and liver parameters

All geese (except the control group) consumed the diet provided. As shown in Figure 1, HSCAS

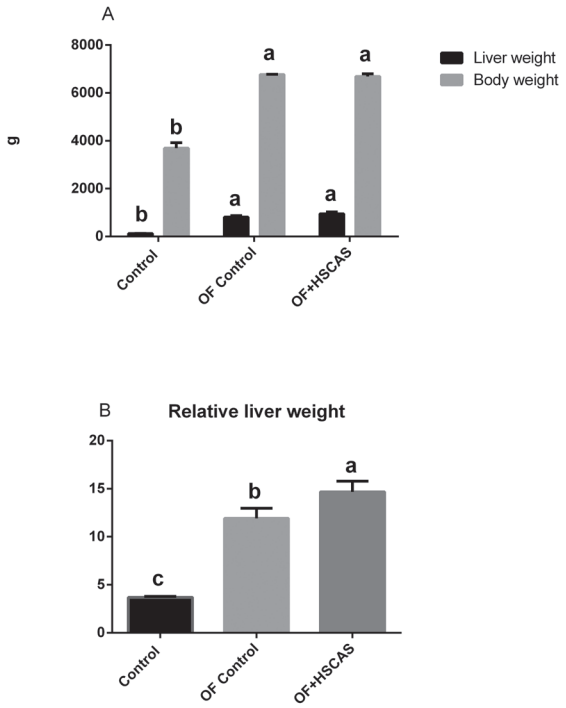

Figure 1 – Effects of HSCAS supplementation on body weight and fatty liver performance of overfed Landes geese.

treatment led to an increase in liver weight, body weight and liver/body weight compared with the control group ($p<0.05$). The HSCAS-treated group also had greater liver weight and liver/body weight compared with the overfeeding control group, which improved by 17.35% (140 g) ($p>0.05$) and 2.28% ($p<0.05$), respectively. No difference was observed in body weight between the geese fed the overfeeding diet without or with HSCAS.

Bacterial communities in Landes geese

The microbial diversity in samples was estimated by calculating the number of OTUs. For each ileal sample, the number of OTUs based on a sequence similarity cutoff of 0.05 was 598 ± 135, with sample coverage of 99.00 ± 0.25%. The average number of sequences was 90,544, and eight different phyla were recorded among 131 different taxa. The Chao1, ACE, and Shannon indices were 1048.46 ± 322.72, 1191.99 ± 408.70, and 3.24 ± 0.27, respectively (Table 1).

Table 1 – Estimators of diversity during overfeeding (OF) in the ileum and ceca of Landes geese

Item	Group			
	Control	OF Control	HSCAS	SEM
Ileum				
Number of OTUs	598	478	985	184.73
Chao1	1048.46	954.16	1778.78	322.72
ACE	1191.99	1206.91	2197.34	408.70
Shannon	3.24	2.80	2.43	0.27
Ceca				
Number of OTUs	579	497	474	43.90
Chao1	630.02	551.73	586.96	42.10
ACE	652.41	567.63	616.39	40.37
Shannon	5.84	4.01	4.22	0.37

ACE, abundance-based coverage estimator; OTU, operational taxonomic unit; SEM, standard error of the mean.

The majority of the diversity in the ileum before OF was represented by *Proteobacteria* sequences (49.4%). The rest of the sequences were composed of *Firmicutes* (21.1%) and *Actinobacteria* (8.3%) OTUs. Other phyla such as *Bacteroidetes* or *Fusobacteria* represented less than 2% of the sequences (Figure 2a). At the class level, the microbiota were mainly composed of *Gammaproteobacteria* (23.69%) and *Alphaproteobacteria* (12.16%) in the phylum *Proteobacteria*; *Clostridia* (5.21%) and *Bacilli* (14.21%) in the *Firmicutes*; and *Bacteroidia* (1.46%) in the *Bacteroidetes* (Figure 2b).

Regarding cecal samples, the number of OTUs based on a sequence similarity cutoff of 0.05 was 579 ±132. The average number of sequences was 64,056, with 10 phyla represented by 167 different taxa. The

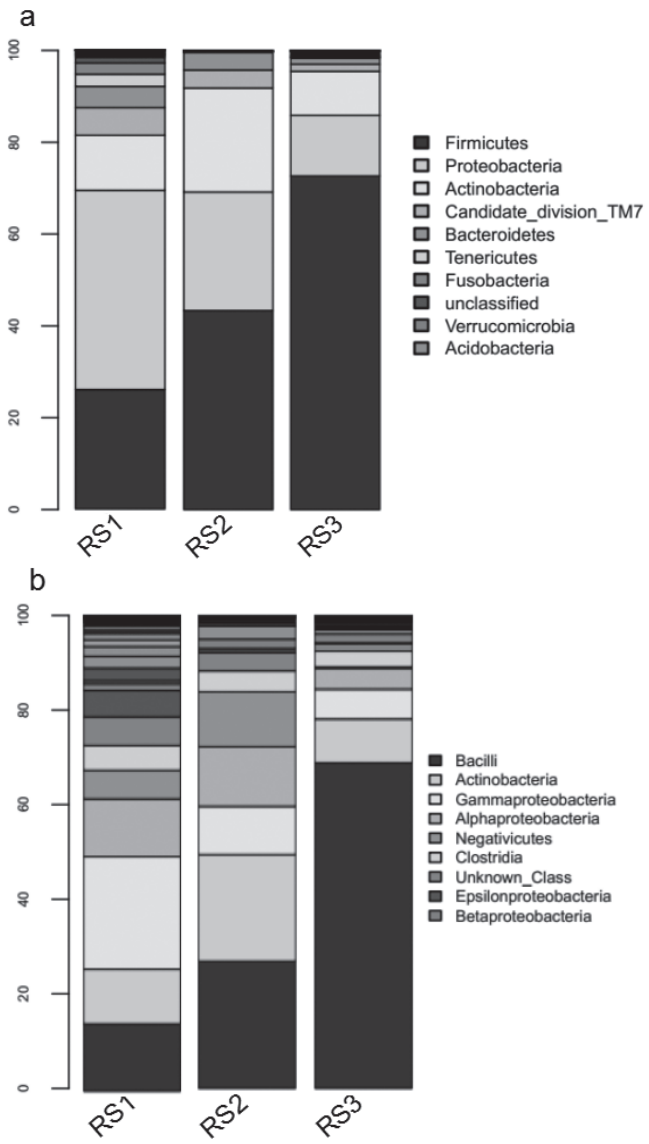

Figure 2 – Percentage contribution of sequences at the phylum (a) and class (b) levels to the total number of sequences in ileum during overfeeding (OF), with or without HSCAS treatment. Non-OF (RS1), OF without HSCAS supplementation (RS2), and OF with HSCAS supplementation (RS3).

Chao1, ACE, and Shannon indices were 630.02 ± 42.10, 652.41 ± 40.37, and 5.84 ± 0.37, respectively (Table 1). The sequences retrieved from the ceca were mainly composed of *Bacteroidetes* OTUs (38.5%). *Firmicutes* sequences amounted to 45.5% of the total, and *Proteobacteria* sequences accounted for 6.6% (Figure 3a). The sequences from two classes, *Bacteroidia* (38.4%) of the phylum *Bacteroidetes*, and *Clostridia* (34.4%) from the phylum *Firmicutes*, were dominant in the ceca (Figure 3b).

Effect of HSCAS on the ileal microbial communities in overfed geese

In samples from HSCAS-overfed geese, indicators showed increased species richness in the ileum and

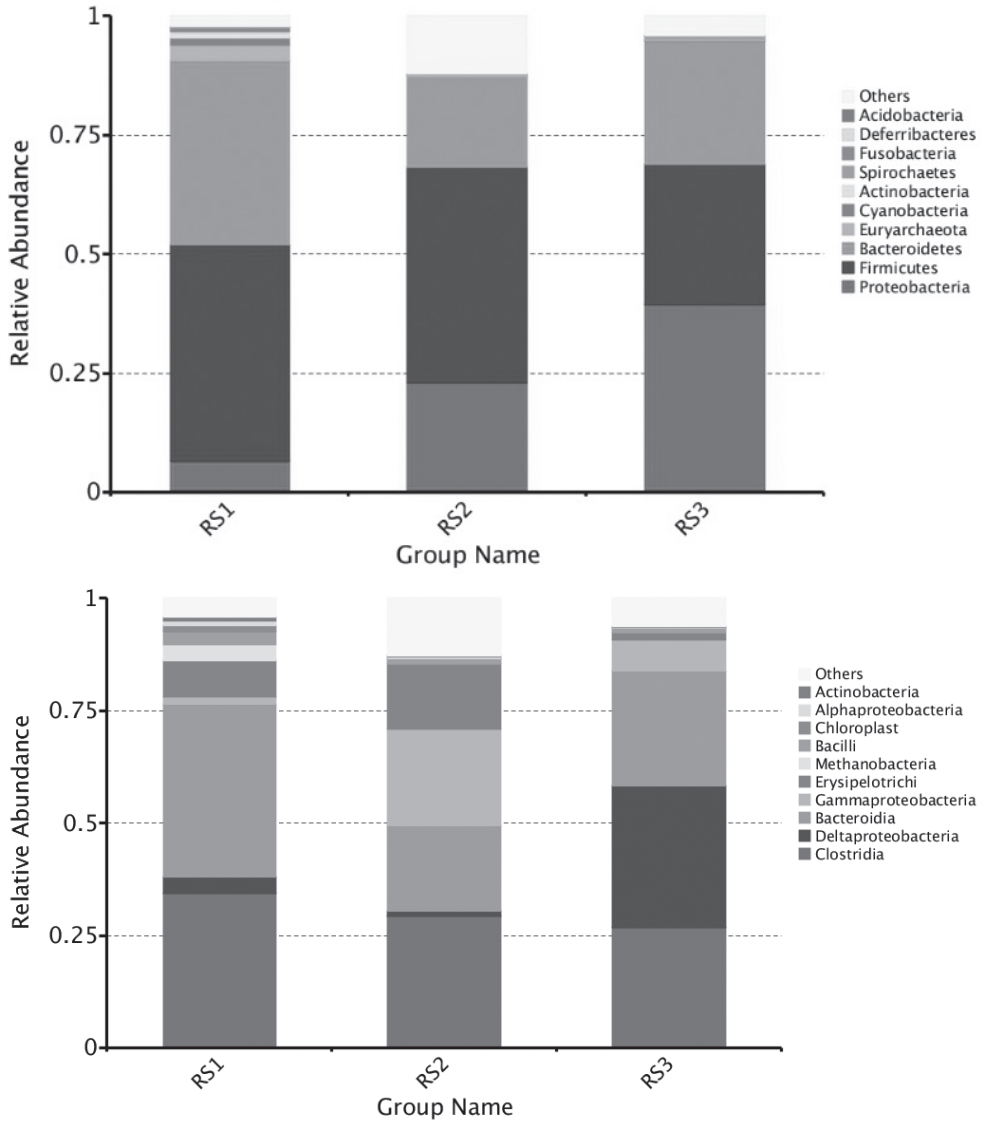

Figure 3 Percentage contribution of sequences at the phylum (a) and class (b) levels to the total number of sequences in cecum during overfeeding (OF), with or without HSCAS treatment. Non-OF (RS1), OF without HSCAS supplementation (RS2), and OF with HSCAS supplementation (RS3).

ceca at end of OF compared to control II. In the ileum, before OF, microbiota were mainly composed of *Gammaproteobacteria* (23.69%), followed by *Bacilli* (14.21%), *Alphaproteobacteria* (12.16%), *Actinobacteria* (11.52%), *Clostridia* (5.21%), and *Bacteroidia* (1.46%), as indicated by the sequencing data (Figure 2a).

At the end of the OF phase, *Bacilli* were most well represented (26.97%) among the sequences, followed by *Actinobacteria* (22.58%), *Gammaproteobacteria* (10.22%), and *Alphaproteobacteria* (12.64%), whereas *Clostridia* represented only 4.34% of the population and *Bacteroidia* were almost nonexistent (0.53%) (Figure 2b). A statistically significant decrease in *Helicobacter* and Pseudoalteromonaswas observed in the sequencing results (p<0.05). After OF, *Actinobacteria*, including *Corynebacterium*; *Gammaproteobacteria*, including *Actinobacillus*; and *Lactobacillus* in *Bacilli* in ileal all increased compared with which before OF (Table 2) (p<0.05).

The addition of HSCAS triggered an increase in representation of the class *Bacilli* from 26.97% without HSCAS to 69.02% with it. This increase coincided with a decrease in *Actinobacteria* from 22.58% to 9.26%, *Gammaproteobacteria* from 10.22% to 6.34%, *Alphaproteobacteria* from 12.64% to 4.39%, and *Clostridia* from 4.43% to 3.17%.

As for the effect of HSCAS on bacterial genera, *Corynebacterium* decreased at the end of OF without HSCAS, and increased dramatically when HSCAS was administered (Table 2). A statistically significant increase in total bacterial load and *Firmicutes* representative,

Table 2 – Statistical effect of overfeeding and addition of HSCAS on different genera in the ileum or ceca of Landes geese.

Item	Group			
	Control	OF Control	HSCAS	*p*-value
Ileum				
Lactobacillus	6.77 ± 0.85[c]	24.01 ± 3.42[b]	54.92 ± 5.36[a]	0.015
Corynebacterium	4.19 ± 1.14[b]	14.65 ± 3.18[a]	5.29 ± 1.10[b]	0.021
Helicobacter	3.39 ± 1.22[a]	0.82 ± 0.31[b]	0.22 ± 0.09[b]	0.048
Actinobacillus	0.50 ± 0.23[b]	5.42 ± 1.06[a]	2.54 ± 1.52[a]	0.048
Pseudoalteromonas	0.85 ± 0.43	0.03 ± 0.01	0.03 ± 0.01	0.069
Ceca				
Bacteroides	30.26 ± 2.13[a]	11.09 ± 3.31[b]	7.01 ± 1.54[b]	0.001
Clostridium	4.68 ± 2.24	0.22 ± 0.10	0.12 ± 0.02	0.078
Desulfovibrio	3.46 ± 1.62	0.12 ± 0.03	0.23 ± 0.02	0.075
Lactobacillus	1.98 ± 0.68[a]	0.18 ± 0.08[b]	0.32 ± 0.24[b]	0.041
Escherichia	0.74 ± 0.30[b]	18.86 ± 5.14[a]	6.18 ± 2.69[b]	0.022

a,b: Mean values within a row with no common superscript differ significantly ($p<0.05$).

including *Lactobacillus*, was observed. *Helicobacter* and *Actinobacillus* in the phylum *Proteobacteria* decreased substantially (Table 2).

Effect of HSCAS on cecal microbial communities in overfed geese

Before OF, the cecal microbiota consisted essentially of *Bacteroidia* (38.4%) and *Clostridia* (34.4%). Some changes were detected during OF, but these two classes remained dominant during OF. After OF, there was a significant increase in *Gammaproteobacteria* (21.5%), concomitant with a decrease in *Bacteroidia* (19.2%) and *Clostridia* (29.4%). These three classes were dominant at the end OF, representing 70% of the total bacterial population (Figure 3b).

Treatment with HSCAS triggered an increase in the *Deltaproteobacteria* class from 0.9% without HSCAS to 31.3% with it. This increase coincided with a decrease in *Gammaproteobacteria* from 21.5% to 6.9%, whereas *Clostridia* decreased only slightly from 29.4 to 26.9% and *Bacteroidia* increased from 19.2% to 25.7%. Treatment with HSCAS did not affect the number of *Bacilli* sequences in ceca. At the end of OF, only a small percentage of *Bacilli* sequences (3.55%) were found in the HSCAS group. In contrast, *Erysipelotrichi* sequences decreased from 14.60% in HSCAS-free overfed birds to 1.65% in HSCAS-fed birds (Figure 3b).

Lactobacillus increased significantly at the end OF with HSCAS. A statistically significant decrease in the total bacterial load and representatives of the *Firmicutes*, including *Clostridium*, was observed. Sequences representing *Bacteroides* and *Escherichia* also decreased markedly (Table 2).

DISCUSSION

The liver is the main organ responsible for *de novo* lipogenesis in avian species (Li *et al.*, 2015). Moreover, the liver is both the main organ for detoxification and the principal toxic target organ (Neeff *et al.*, 2013). The best grade fatty liver is produced in France, where government regulations require stricter quality controls in foie gras production (Skippon, 2013; Gourmetfoodstore, 2016). For the sake of safety and quality of foie gras as well as good health of birds, the adverse effects should be avoided in the management of geese liver production.

Aluminosilicates (mainly zeolites, HSCAS) are known as feed additives used for multiple purposes in the poultry industry due to their capabilities as mycotoxin adsorbents and other qualifications. The histopathology of liver showed that lesions attributed to aflatoxicosis were ameliorated by adding 0.2% HSCAS to the contaminated foods (Zhao *et al.*, 2010). This substance binds to AF molecules in the gastrointestinal tract, making them unavailable for adsorption and consequently alleviating aflatoxicosis (Liu *et al.*, 2011).

In the present study, overfeeding led to an increase in relative weight of liver compared with the negative control ($p<0.05$). Overfeeding of HSCAS-treated geese exhibited benefit effects on fat deposition compared to those of overfeeding alone, with the liver percentage relative to body weight increasing 2.50% ($p<0.05$) at the end of the overfeeding period. Su *et al.*, (2009) investigated the impact of betaine supplementation on the fatty livers in geese and found that the betaine diet increased liver weight by more than 100g (Su *et al.*, 2009). Betaine has been considered an ideal hepatic protectant against both alcoholic (Ji & Kaplowitz, 2003;

Day & Kempson, 2016) and non-alcoholic steatosis (Neuschwander-Tetri, 2001; Chen *et al.*, 2015).

With respect to HSCAS, it has reduced the incidence and severity of the hepatic histopathology changes associated with aflatoxicosis (Chen *et al.*, 2014). In the present study, HSCAS supplementation increased the relative liver weight. This suggests that lipogenesis and fatty liver production was thus improved in the HSCAS-treated geese. The HSCAS, which induces beneficial effects on the apparent nutrient retention, may be associated with decreasing lesions in gastrointestinal tract through the effective absorption of aflatoxins (Liu *et al.*, 2011). This may explain the improved liver weight and liver to body weight. A healthy gut not only affects nutrient utilization in the birds, but also has exerts considerable influence on the microbiota composition of the bird, which was observed in the ileal and cecal microbiota of geese in this study.

In other studies on the intestinal microbiota of birds and mammals, *Firmicutes* and *Bacteroidetes* were identified as the major phyla, suggesting their importance in metabolism and host physiology (Kohl, 2012). In contrast, the current study showed *Firmicutes* and *Proteobacteria* to be the major phyla. *Firmicutes* was the second most common phylum after *Proteobacteria* in ileal samples, based on HiSeq2500 sequencing. *Firmicutes* was also the major phylum in feces of Canada geese (Lu *et al.*, 2009) and Muscovy ducks (*Cairina moschata*)(Vasai *et al.*, 2014a), whereas *Bacteroidetes* are less abundant in some avian species (Lu *et al.*, 2007). In contrast to mule ducks, where obligate anaerobes (class *Clostridia* and *Bacteroidia*) dominated in both ileal and cecal samples (Vasai *et al.*, 2014b), in geese, obligate anaerobes (class *Clostridia* of *Firmicutes* and *Bacteroidetes*) dominated the ceca samples, and facultative anaerobes (*Gammaproteobacteria*, *Bacilli*, and especially *Lactobacillus*) were dominant in the ileum. Higher numbers *Clostridia* sequences were observed in ducks than in geese, suggesting that bacterial digestive metabolism in geese and ducks may be somewhat different.

OF increased the relative abundance of *Firmicutes*, and especially *Lactobacillus*, in ileal samples from geese, as previously described in mule, Pekin, and Muscovy ducks (Vasai *et al.*, 2014a; Vasai *et al.*, 2014b). The *Lactobacillus* group, members of the class *Bacilli*, are known as amylolytic bacteria and frequently increase in pigs fed with diets rich in starch (Regmi *et al.*, 2011). According to the high-throughput sequencing results, *Lactobacillus*, *Actinobacteria*, and *Corynebacterium* numbers increased; however, for *Corynebacterium* and *Actinobacteria*, this increase was reversed by HSCAS supplementation. *Helicobacter*, in contrast, decreased with HSCAS supplementation.

HSCAS increased the diversity indexes of samples from the ileum, whereas in the ceca, an increase in these indexes only occurred in comparison with control II, while they decreased relative to control I. In broiler chicks, the addition of 0.5% HSCAS to an aflatoxin B1 diet significantly improved feed intake and weight gain (Gowda *et al.*, 2008). In the current study, the weight of the fatty liver, as well *Firmicutes/Bacteroides* ratios, increased in comparison with those of overfed controls, but no significant effect on growth performance was observed (data not shown).

Feeding with HSCAS triggered an increase in ileal *Bacilli* numbers and an increase in sequences representing the phylum *Firmicutes*. The dominant sequences similar to those of *Bacilli* were most abundant in waterfowl, Canada geese, and gulls. On average, sequences similar to those of *Bacteroidetes* represent a smaller fraction of the avian fecal community, particularly in waterfowl (7.1%) (Lu *et al.*, 2009).

Thus far, there is little information on the effect of HSCAS on the diversity of goose fecal microbiota, although it has been hypothesized that HSCAS has a health impact on waterfowl gut microbial communities. In aquatic species, dietary supplementation with Azomite (an HSCAS product) enhances innate immunity and disease resistance in *Oreochromis mossambicus* against *Aeromonas hydrophila* (Musthafa *et al.*, 2016).

In our study, the HSCAS diet resulted in an increased proportion of 16S rRNA sequences in the *Lactobacillus* group, whereas the percentages of *Proteobacteria*, including *Corynebacterium*, *Helicobacter* and *Altererythrobacter* decreased. The relative abundance of *Lactobacillaceae* increased substantially, leading to a decrease in most other families (Vasai *et al.*, 2014a). Previous studies showed that dietary supplementation with *Lactobacillus*-based probiotics increased gastrointestinal *lactobacilli* counts and decreased *coliforms* numbers (Hassan & Ryu, 2012). Vasaï reported that the addition of *Lactobacillus sakei* triggers major changes in the ileum, whereas ceca were not affected. *Lactobacillus sakei* decreased the relative abundance of *Bacteroides* at mid-OF and the relative abundance of *Enterobacteria* in the *ileum* at the end of OF (Vasai *et al.*, 2014b). The above-mentioned results are in agreement with a study in mice, in which probiotic treatment ameliorated metabolic syndrome symptoms, as well as increased *Lactobacillus* spp. and *Bifidobacterium* spp. and decreased *Clostridiaceae*

spp., *Akkermansia* spp., and *Escherichia coli* (Park *et al.*, 2013).

The decrease in these potentially pathogenic bacteria was simultaneous with an increase in *Bifidobacterium* spp. and *Lactobacillus* spp./*Enterococcus* spp. (Rawski *et al.*, 2016). In poultry species, dietary supplementation with *Lactobacillus*-based probiotics suppressed the growth of potentially pathogenic bacteria such as *Clostridium*, *Escherichia coli*, and *Salmonella enterica*, as determined by culture-dependent methods (Vasai *et al.*, 2014b). Supplementation with HSCAS resulted in a decrease in *Helicobacter* and *Actinobacillus*, suggesting that HSCAS can protect against potential pathogens. The number of sequences representing *Corynebacterium* also decreased significantly with HSCAS supplementation, suggesting some level of antagonism between HSCAS and these bacteria. Because a decrease in the total bacterial load in ileum was observed at the end of OF, it is possible that HSCAS inhibited growth of several other bacteria.

The cecal samples showed a very different pattern than the ileal samples in response to HSCAS. First, supplementation with HSCAS altered the microbial communities, as determined by the nonmetric multidimensional scaling analysis and bacterial composition. In chickens, *Clostridia* and *Bacteroides* (obligate anaerobes) were predominant in cecal samples, whereas *Lactobacillus* spp. (facultative anaerobes) was an important component of the ileal microbiota (Lu *et al.*, 2003). A study of two parental genetic types (Pekin and Muscovy ducks) showed that cecal microbiota was mainly composed of *Firmicutes* and *Bacteroidetes* (Vasai *et al.*, 2014a). Both *Lactococcus* and *Paenibacillus* spp. have been isolated from poultry ceca or feces, so they can be considered normal members of the avian gut microbiota (Lu *et al.*, 2009). The complex anaerobic environment may be refractory to changes in the microbial community (Vasai *et al.*, 2014b). In this study, HSCAS feeding had an effect in ceca. Treatment with HSCAS increased representation of the *Deltaproteobacteria* class from 0.9% without HSCAS to 31.3% with it. This increase coincided with a decrease in *Gammaproteobacteria* from 21.5% to 6.9%. OF led to a decrease in *Bacteroides*, *Clostridium* and *Escherichia* numbers at the end of OF; however, for *Clostridium* and *Escherichia*, this decrease was reversed by HSCAS supplementation.

In addition, we observed an increase of the *Erysipelotrichi* class after OF in Landes geese, which was reduced by HSCAS treatment. Previously, the class *Erysipelotrichi* was reported to be overabundant in the obese state (Turnbaugh *et al.*, 2009). Overgrowth of members of the class *Erysipelotrichi* was observed after feeding obese mice but not lean mice after feeding with a high-fat diet (Upadhyay *et al.*, 2012). This implicated *Erysipelotrichi* as a species that might contributed to metabolic disease in a host on a high-fat diet (Upadhyay *et al.*, 2012); however, overgrowth of this class of bacteria was reversed by HSCAS in the current study. Thus, HSCAS may have the beneficial effect of restoring balance to gut microbes.

Bacteroides are predominant in intestines because of their ability to utilize polysaccharides (Ravcheev *et al.*, 2013). An imbalance in the gut microbiota, known as dysbiosis, is usually associated with a sudden increase in the abundance of facultative anaerobic *Gammaproteobacteria*, particularly *Enterobacteriaceae*, which is characteristic of gut malfunction and intestinal inflammation (Winter *et al.*, 2013). HSCAS reduced the number of *Gammaproteobacteria* and increased that of *Bacteroidia*. HSCAS can be inferred to play a role in reducing inflammation. In the *Proteobacteria*, the class *Gammaproteobacteria* has been shown to increase its numbers with increasing cholic acid concentrations (Islam *et al.*, 2011). The relative abundance of members of the order *Enterobacteriales* (class *Gammaproteobacteria*) have been reported to significantly increase in rats fed a high-fat diet, and *Enterobacteriaceae* are known to be highly tolerant of bile acid (de La Serre *et al.*, 2010). A high-fat diet enhances bile secretion to facilitate lipid digestion (Reddy, 1981). Cholic acid feeding simplified the composition of the microbiota, with the overgrowth of several bacteria in the classes *Clostridia* and *Erysipelotrichi* (Islam *et al.*, 2011). This effect may explain the increase in *Gammaproteobacteria* and decrease in *Bacteroidia* in the ceca of overfed geese.

CONCLUSION

In conclusion, dietary HSCAS supplementation promoted liver growth performance in overfed Landes geese. This study, using high-throughput sequencing based on 16S rRNA in ileal and cecal samples, provides information on the microbial communities in Landes geese and the effect of administering HSCAS on the abundance of major groups during OF. The gut microbial community in geese is dominated by *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, and OF modifies the bacterial communities primarily in cecal samples, whereas HSCAS shows an important effect on ileal samples. The increase in *Lactobacillus* during

OF with the addition of HSCAS as a prebiotic, may improve the bird's health and therefore be of great interest to the poultry industry.

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CONFLICT OF INTEREST

All authors read and approved the findings of the study. None of the authors have any conflicts of interest. We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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