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Alteration in bile acids profile in Large White pigs during chronic heat exposure

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ABSTRACT

Bile acids (BAs) are critical for cholesterol homeostasis and new roles in metabolism and endocrinology have been demonstrated recently. It remains unknown whether BA metabolism can be affected by heat stress (HS). The objective of this study was to describe the shifts in serum, hepatic and intestinal BA profiles induced by chronic HS. Twenty-seven Large White pigs weighing 40.8 ± 2.7 kg were assigned to one of the three treatments: a control group (CON, 23 °C), a HS group (33 °C), or a pair-fed group (PF, 23 °C and fed the same amount as HS group) for 21 d. The concentrations of taurine-conjugated BAs (TUDCA and THDCA in serum and TCDCA, TUDCA, THDCA and THCA in liver) were decreased in HS and PF pigs. However, in HS pigs, a reduction in taurine-conjugated BAs (TCBA) correlated with decreased liver genes expression of BA synthesis, conjugation and uptake transport. BA regulated-genes (FXR, TGR5 and FGFR4) in HS pigs and TGR5, FGFR4 and KLβ in PF pigs were down-regulated in liver. In ileum, total BAs and glycoursodeoxycholic acid concentrations were higher in HS pigs than other groups and PF group, respectively ($P < 0.05$). TCBA ($P = 0.01$) and tauroursodeoxycholic acid ($P < 0.01$) were decreased in PF group. BA transporters (OSTα and MRP3) were up-regulated in HS pigs compared with CON and PF pigs, respectively ($P < 0.01$). In cecum, ursodeoxycholic acid was higher in HS ($P = 0.02$) group than CON group. The expression of apical sodium-coupled bile acid transporter ($P = 0.04$) was lower in HS pigs than CON pigs, while OSTβ ($P < 0.01$) was greater in HS group than PF group. These results suggest that chronic HS suppressed liver activity of synthesis and uptake of TCBA, at least in part, which was independent of reduced feed intake.

1. Introduction

Heat stress (HS) influences many productive traits and increases economic loss in pigs (Pollmann, 2010). In heat-stressed pigs, feed intake is reduced by as much as 40–50% to reduce heat production, which severely impairs the growth performance of pigs (Pearce et al., 2013a). Also heat exposure causes profound damages in the intestinal barrier functions (Liu et al., 2016), indicated by shortening of the villi, and increased permeability (Gabler et al., 2018; Pearce et al., 2013a). It is hypothesized that in heat-stressed animals leaky gut leads to insulin sensitivity because of endotoxemia (Sanz Fernandez et al., 2015; Xin et al., 2018). Although feed intake is inadequate in heat-stressed pigs, lipolytic activities are reduced but lipid deposition is promoted both *in vivo* and *in vitro* (Baumgard and Rhoads, 2013; Qu et al., 2015; Sanders et al., 2009; Torlińska et al., 1987). Recently, we found 21 days of heat exposure at 33 °C can temporally increased serum circulating

Abbreviations: BAs, bile acids; TBA, total BAs; PBA, primary BAs; SBA, secondary BAs; TCBA, taurine-conjugated BAs; GCBA, glycine-conjugated BAs; CA, cholic acid; CDCA, chenodeoxycholic acid; HCA, hyocholic acid; LCA, lithocholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TLCA, taurolithocholic acid; TCDCA, taurochenodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; THDCA, taurohyodeoxycholic acid; TDCA, taurodeoxycholic acid; THCA, taurohyocholic acid; CYP7A1, cholesterol 7α-hydroxylase 1; CYP27A1, sterol 27-hydroxylase; CYP8B1, sterol 12α-hydroxylase; BAAT, bile acid-CoA:amino acid N acyltransferase; CCK, cholecystokinin; BACS, bile acid-CoA synthase; ASBT, apical sodium-coupled bile acid transporter; OSTα/β, organic solute transporters α/β; IBABP, ileum bile acid-binding protein; MRP2/3, multidrug resistance protein 2/3; BSEP, bile salt export pump; NTCP, sodium-taurocholate cotransporting polypeptide; OATP1B3, organic anion transporting peptides 3; FXR, farnesoid X receptor; RXR, retinoid X receptor; TGR5, membrane-bound G-protein coupled receptor; GLP-1, glucagon-like peptide-1; FGF19/15, fibroblast growth factor19/ 15; SHP, small heterodimer partner; FGFR4, fibroblast growth factor receptor 4; KLβ, Klotho beta

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cholesterol in growing pigs on day 3, while pair-fed decreased blood cholesterol after 21 days (Wen et al., unpublished data). This phenomenon has also been reported by Pearce et al. (2013b). So far, it is not completely known how cholesterol homeostasis is regulated when nutrients and energy intake is severely reduced in heat-stressed pigs.

Bile acids are synthesized and conjugated exclusively from cholesterol in the liver (Angelin and Carlson, 2010; Kortner et al., 2013). The conversion of cholesterol to the BAs is the major route for cholesterol excretion (Chiang, 1998; Cohen, 2008; Siperstein et al., 1952). As an emulsifier, BAs are essential for fat digestion and lipid-soluble vitamins absorption in the intestine (Russell and Setchell, 1992). Furthermore, BAs can coordinate glucose, lipid and energy homeostasis through the farnesoid X receptor (FXR) and the membrane-bound G-protein coupled receptor (TGR5) (Houten et al., 2006; Lefebvre et al., 2009). Bile acids interact with TGR5 to stimulate glucagon-like peptide-1 (GLP-1) secretion in the large intestine to regulate feed intake (Gutzwiller et al., 1999; Katsuma et al., 2005; Thomas et al., 2009).

Primary BAs are synthesized in pericentral hepatocytes through a series of sterol ring hydroxylations and side chain oxidation steps. They are conjugated with glycine or taurine under bile acid-CoA synthase (BACS) and bile acid-CoA:amino acid N acyltransferase (BAAT) before being secreted into the gallbladder for storage. Upon the signal of cholecystokinin (CCK), gallbladder contracts to release stored BAs into the duodenum during a meal. Under the deconjugation and 7α-dehydroxylation activities of gut microbes, conjugated BAs are converted into free BAs and primary BAs are converted to secondary BAs, respectively (Midtvedt, 1974; Ridlon et al., 2006). About 95% of the BAs are reabsorbed in the intestine, especially at the distal ileal section, to save the total *de novo* hepatic BAs synthesis (Dawson and Karpen, 2015; Russell, 2003). Bile acid biosynthesis is tightly controlled by enterohepatic circulation. When BAs pass through the ileal section, ileocytes synthesize fibroblast growth factor 19 (FGF19; FGF15 in rodents), which interacts with fibroblast growth factor receptor 4 (FGFR4)/Klotho beta (KLβ), complex to negatively regulate BA synthesis in the liver (Holt et al., 2003). In addition, BAs can interact with FXR to induce the small heterodimer partner (SHP), suppressing the expression of BA synthetases as well as cholesterol synthesis (Lu et al., 2000; Zhu et al., 2012).

In view of the close relationship between BA and cholesterol metabolism, we hypothesized that heat stress can alter BA metabolism, which contributes to the up-regulated serum cholesterol in the heat-stressed pig (Wen et al., unpublished data). The effects of HS on metabolites (including BAs) have been examined by metabolomics (Ippolito et al., 2014; Wang et al., 2016). However, there is no literature about the effects of heat stress on individual BA as well as on the BA composition. Therefore, the current study aimed to investigate hepatic biosynthesis, intestinal transport of BA and abundance of bacteria related to BA metabolism in growing pigs experiencing chronic heat exposure.

2. Materials and methods

2.1. Animals and experimental design

All experimental procedures involving animals used in this experiment were approved by the Laboratory Animal Care Advisory Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (IASCAAS).

To distinguish genetic differences, the experiment adopted a completely random block design, which considered the litter as the block. A total of 27 castrated male Large White pigs from 9 litters (3 pigs/litter) with an average body weight of 40.8 ± 2.7 kg, were allotted to one of the three treatments: a control group for which the environment was maintained at 23 °C (CON, with *ad libitum* water and feed intake), a heat stress group, continuously kept at 33 °C (HS, with *ad libitum* water and feed intake), or a pair-fed group (PF, *ad libitum* access to water) kept at 23 °C but given the same amount to the feed consumed on the previous day by the HS group, for 21 days. Pair-fed was conducted to determine the effects of a reduced nutrient intake. The experiment was executed over 3 consecutive periods and 9 pigs from 3 litters were assigned into 1 of the 3 groups per period.

Table 1
Composition and nutrient levels of the basal diet (air-dry basis).

Items	Percentage	Items	Percentage
Ingredients		Nutrient levels[2])	
Corn	62.55	DE (Mcal/kg)	3.23
Soybean meal	27.00	CP	18.00
Wheat bran	5.00	Lys	1.03
Soybean oil	1.00	Ca	0.95
Limestone	1.50	P	0.74
$CaHPO_4$	1.5		
NaCl	0.3		
L-Lysine-HCl	0.15		
Premix[1)]	1.00		
Total	100		

[1)] Premix provided the following per kg of diets: vitamin A 8250 IU, vitamin D3 825 IU, vitamin E 80 IU, vitamin K 4.25 mg, vitamin B1 1.02 mg, vitamin B2 5.20 mg, vitamin B6 2.04 mg, vitamin B12 2.5 mg, biotin 0.2 mg, pantothenic acid 15.3 mg, nicotinic acid 35.7 mg, folic acid 2 mg, Fe ($FeSO_4$) 266 mg, Cu ($CuSO_4$) 200 mg, Zn ($ZnSO_4$) 285 mg, Mn ($MnSO_4$)78 mg, I (KI) 0.8 mg, Se (Na_2SeO_3) 0.3 mg, choline chloride 600 mg.

[2)] Estimated using the NRC (2012) individual dietary ingredients.

The animal rooms were illuminated with a 16 h light cycle (light during 6.00 a.m.–10.00 p.m.) with a relative humidity of 55% ± 5%. The diet consisted primarily of ground corn and soybean meal and was formulated to meet or exceed nutrient requirements suggested by the NRC (2012). No antibiotics were included in the diets (Table 1). Prior to the experiment, animals were allowed to acclimate to their pens for a week for adaption and were reared at 23 °C. The data of average daily feed intake, average daily gain, rectal temperatures and respiration rates have been reported in Xin et al. (2018). Compared with CON pigs, HS pigs showed a 55.6% reduction in average daily feed intake, 41.2% reduction in average daily gain (41.2%). They increased rectal temperatures by 0.8 °C and respiration rates by 2 folds. At the end of the trial (21 d), all the pigs were slaughtered via electrical stunning followed by exsanguination. Feed was withheld from the pigs 12 h before slaughter. Blood samples were taken via the jugular vein before they were sacrificed. Blood was collected into vacuum tubes, subsequently clotted and centrifuged at 3000 rpm for 10 min at 4 °C. Then, serum was obtained, aliquoted and stored at −80 °C for BAs quantification. Liver samples were stored at −80 °C for BAs and real time PCR (q-PCR) measurements. Mucosae of ileum and cecum were scraped and then snap frozen in liquid nitrogen followed by storage at −80 °C for mRNA analysis. Digesta from ileum and cecum were collected for BAs and bacteria quantifications.

2.2. LC-MS/MS to quantify bile acids in serum and liver as well as intestinal digesta

To extract BAs in the serum, a volume of 100 μL serum was mixed with an equal amount of pre-cold sodium acetate buffer (50 mM, pH 5.6) and triple ethanol. The mixture was then vortexed for 2 min, allowed to stand for 30 min at 4 °C, and centrifuged at 20,000 g for 20 min. The supernatant was diluted four times with sodium acetate buffer and applied to a Bond Elute C18 cartridge (500 mg/6 ml; Harbor city, CA) which has been pre-activated by 5 ml methanol. The cartridge was washed with 25% ethanol and eluted with 5 mL of methanol. Next, the solvent was removed under nitrogen gas, the residue was reconstituted with 1 ml of methanol (Fang et al., 2018). Precipitated solids were then removed by filtration using a 0.45 μm Millipore filter (Millex®-LG; Billerica, MA). To extract liver BAs, appropriate

Table 2
Primers for bile acids-related genes and bacteria genes.

Gene	Gene Bank ID	Sequences, forward/reverse 5′-3′	Amplicon size (bp)
Bile acids biosynthesis			
CYP7A1	NM_001005352.3	GAAAGAGAGACCACATCTCGG GAATGGTGTTGGCTTGCGAT	123
CYP27A1	NM_001243304.1	ACTGAAGACCGCGATGAAAC CAAAGGCGAATCAGGAAGGG	106
CYP8B1	NM_214426.1	CCGGAAGAATATGTTGGAAT AAGTCTAGTTTTCTCTTCGC	150
BAAT	XM_003122044.4	GGCTGATGATCCGAGAAGGG ATGCCCCCAAACAAGTCGAT	94
BACS	XM_005658625.1	CTGGCTCCCTGCCTATGCT GAACGTGCTTGTGGTCTCCAA	70
Bile acids transports			
BSEP	XM_003133457.4	TTGGAGCAGGTTGGAACACC CACATTTCTTCACGCTGCGA	145
OATP1B3	XM_021091087.1	GGAATGATTGGTCCGATCCTTGGC AACCAAGCCACCAAGCACCAAC	139
NTCP	XM_001927695.4	ACTTTCGGAAACCTAAGGGACT AAGAGCTTGCCCAGTGCAAAG	88
ASBT	NM_001244463.1	TGGATCTGAGCATCAGCATGAC GGCACAGCGGCATCATTC	66
IBABP	NM_214215.2	GTGAACAGCCCCAACTACCA CCACTAGCTCATGCCAGCTT	119
OSTα	NM_001244266.1	TGTACAAGAACACTCGCTGC GAACACACACACTATCGTGGG	80
OSTβ	XM_013991788.1	CTGGTGGCTGTGGTGGT AAAGTCTCCCTTAGGATGGT	167
MRP2	DQ530510.1	GAACAGGTTTGCTGGCGATATT GCCAGGAGCGCAAAGACA	65
MRP3	XM_003131575.5	TGGACAAAGGGACAATAGCTGAGT TGGCCATCCCGTAGAAGATG	78
Bile acids receptors			
FXR	NM_001287412.1	ATACAACAGTGTTCCGTTTC AGAGTCTCAGCAGGCATT	144
TGR5	XM_013984487.1	AAGCCCAAGATGACACCCAA CCAGGAGCAGACTCAGGAAGAA	187
Bile acids signaling			
SHP	AH014861.3	GCCTACCTGAAAGGGACCAT CAACGGGTGTCAAGCCTTTA	126
FGFR4	XM_003123682.5	GCTCAGAGGTGGAGGTCCTA GCCTGCCAGACAGGTGTATT	74
KLLβ	XM_003482367.4	GCACCGAGTGGAAGGAGTT TGCCAGTAGGAAGGATTG	150
GLP-1	NM_001256594.1	CAGTGCAGAAATGGCGAGAA GGTGGAGCCTCAGTCAGGAA	61
Target bacteria			
Total bacteria	MK616037.1	ACTCCTACGGGAGGCAGCAG GGACTACHVGGGTWTCTAAT	464
Bacteroides	KF374939.1	CATGTGGTTTAATTCGATGAT AGCTGACGACAACCATGCAG	120
Clostridium	MG462904.1	TGAAAGATGCCATCATCATTCAAC GGTACCGTCATTATCTTCCCCAAA	283
Enterococcus	CP030110.1	GAGAAATTCCAAACGAACTTG CAGTGCTCTACCTCCATCATT	93
Lactobacillus	JF923643.1	GAGGCAGCAGTAGGGAATCTTC GGCCAGTTACTACCTCTATCCTTCTTC	126

modification methods were used described by Yang et al. (2017). 100 mg liver tissue was homogenized in 1 ml physiological saline. The supernatant was obtained by centrifugation at 2500 rpm for 10 min. Two hundred microliter liver homogenate was extracted use the same method as described above. Finally, the resulting supernatant was used for LC-MS/MS analysis and the assay conditions have been reported previously (Fang et al., 2018). Bile acids in the intestinal digesta were extracted as previously described (Fang et al., 2018).

2.3. RT-qPCR analysis to quantify bile acid-related genes

The impact of heat exposure on critical genes related to BA synthesis, transportation and signaling were assessed. Primers (Table 2) were characterized by amplification efficiency analyses and agarose gel electrophoresis. Total RNA was isolated from liver or intestinal mucosal scrapings from ileum and cecum using Qiagen kits (RNeasy Mini Kit, Cat # 74104). RNA integrity and concentration were assessed via 2% agarose gel electrophoresis and microspectrophotometer (Nanodrop, Technologies, Wilmington, DE). First-strand cDNA was synthesized by using the High-Capacity cDNA Archive kit (Takara RR047A, China) according to the manufacturer's instructions. Approximately 1 μg of RNA with an average A260/A280 of 1.9 were transcribed. qPCR was performed in a Bio-Rad CFX 96 System using SYBR Green Master Mix (Applied Bio-Rad). The comparative C_T method ($2^{-\Delta\Delta Ct}$) was used to calculate the gene expression values using β-actin as a housekeeping gene.

2.4. Bacteria quantification by qPCR

Bacteroides, *Clostridium*, *Lactobacillus* and *Enterococcus* as the major gut microbes participating in BAs deconjugation and secondary BAs synthesis (Ridlon et al., 2016), were quantified by qPCR. Plasmids containing 16S rRNA fragments of *Bacteroides*, *Clostridium*, *Lactobacillus* and *Enterococcus* were cloned as following. Fragments 16S rRNA were amplified with PCR and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Each purified fragment was cloned into pMD18-T vector (TaKaRa, Beijing, China), followed by transformation into competent *E. Coli* Top 10 cells (Tiangen, Beijing, China). Plasmids were extracted with a commercial plasmid mini kit (Majorbio, Shanghai, China) and the concentration of each plasmid was measured at 260 nm (NanoDrop 2000; Thermo Fisher Scientific, USA). The plasmids obtained were sequenced to validate identity of bacteria. Ten-fold dilutions of each plasmid from 10^2 to 10^6 copies were performed to generate standard curve for absolute quantification.

Microbial DNA was extracted from digesta from ileum and cecum samples using the EZNA™ Soil DNA kit (D5625-02, Omega Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer's protocol. qPCR was performed to quantify *Bacteroides*, *Clostridium*, *lactobacillus* and *Enterococcus* on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). Primers are listed in Table 2. The PCR reaction was performed in a total volume of 20 μL using the ChamQ SYBR Green PCR Core Reagents kit (Vazyme Biotech Co.,Ltd. Nanjing, China). Each reaction included 16.5 μL ChamQ SYBR Color qPCR Master Mix (2X), 0.8 μL of each primer (5 μM) and 2 μL of the template DNA. The cycling conditions were as follows: 95 °C for 5 min, and 40 cycles at 95 °C for 5 s and 60 °C for 30 s.

2.5. Statistical analysis

All the statistical analyses were performed using the JMP10.0 (SAS Institute, Inc., Cary, NC). The one-way analysis of variance (ANOVA) and Duncan's multiple comparison were used to compare study subgroups. Data were presented as mean and SEM and a P value < 0.05 was considered significant.

3. Results

3.1. Serum bile acids profile

Unconjugated BAs, including chenodeoxycholic acid (CDCA, 24.78%), hyocholic acid (HCA, 11.31%) and ursodeoxycholic acid (UDCA, 25.80%) were the predominant BAs in serum, accounting for 61.9% of total BAs (TBA). TCBA accounted for 4.3% and glycine-conjugated BAs (GCBA) accounted for 33.8% of serum TBA (Fig. 1A). After 21 d of heat exposure, TBA, primary BAs (PBA), secondary BAs (SBA) and GCBA did not differ among the three groups. However, TCBA in the HS and PF groups were decreased by 44% ($P = 0.03$) and 42% ($P = 0.02$), respectively, compared with the CON group. Among the four TCBAs, tauroursodeoxycholic acid (TUDCA, $P = 0.025$) and taurohyodeoxycholic acid (THDCA, $P = 0.03$) were reduced in the HS and PF pigs (Fig. 1A).

3.2. Liver bile acids composition

The liver is the critical site for BAs synthesis and secretion. In the liver, GCBA such as glycochenodeoxycholic acid (GCDCA, 61.69%) and glycoursodeoxycholic acid (GUDCA, 32.77%) played a leading role, which constituted more than 94% of TBA. TCBA (5.32%) and unconjugated BAs (0.22%) were relatively minor components (Fig. 1B). Taurochenodeoxycholic acid (TCDCA, TUDCA, THDCA and taurohyocholic acid) were decreased in the liver of HS and PF groups and therefore the TCBAs was lower than the CON group ($P = 0.02$, Fig. 1B). No differences were found in other BAs among different groups in heat exposure for 21 d.

3.3. Intestine bile acids composition

Unconjugated BAs accounted for 65.86 and 90.16% of TBA in ileum and cecum, respectively. However, PBA (CDCA; 20.37% and HCA; 29.22%) constituted the largest portion in the ileum but SBA (LCA; 26.61% and UDCA; 32.58%) in the cecum. PBA (49.65–30.92) and conjugated BAs (34.14–9.84%) decreased, whereas secondary BAs increased (16.21–59.24%) between the ileum and cecum (Fig. 1C and D). Under the HS condition, in the ileum, TBA ($P = 0.01$) was greater in HS pigs than the CON and PF groups and GUDCA ($P = 0.026$) was greater in the HS group than the PF group. In the PF group, TCBA was lower than the CON ($P = 0.003$) and HS ($P = 0.009$) groups (Fig. 1C), where TUDCA ($P < 0.01$) was reduced in the ileum. The BAs composition of the cecal pool was not changed significantly among the three groups and only UDCA was higher in the HS ($P = 0.02$) group than the CON group (Fig. 1D).

3.4. Bile acids synthetases and transporters in liver

Cholesterol 7α-hydroxylase 1 (CYP7A1) and sterol 27-hydroxylase (CYP27A1) initiate the classical and alternative BAs synthesis pathways in the liver (Russell and Setchell, 1992). In comparison to the CON group, CYP7A1 and sterol 12α-hydroxylase (CYP8B1) remained unchanged in the HS group, but CYP27A1 was down-regulated by 45% ($P = 0.01$). BAAT and BACS, two genes coding enzymes catalyzing BAs conjugation, were also down-regulated in the HS group by 41% ($P < 0.01$) and 42% ($P < 0.01$), respectively (Fig. 2A). Liver BA transporters, sodium-taurocholate cotransporting polypeptide (NTCP), organic anion transporting peptides 3 (OATP1B3) and bile salt export pump (BSEP) were down-regulated in the HS pigs ($P < 0.01$, Fig. 2B). In the PF group, only CYP27A1 ($P = 0.02$) was lower than the CON group (Fig. 2A).

3.5. Bile acids transporters in intestine

In comparison to the CON group, in the ileum, chronic heat exposure significantly up-regulated the expression of organic solute transporters α (OSTα; 39%, $P < 0.01$), a BA basolateral transporter (Fig. 3A). Multidrug resistance protein 3 (MRP3) was up-regulated in HS pigs when compared with PF pigs ($P < 0.01$, Fig. 3A).

In the cecum (Fig. 3B), apical sodium-coupled bile acid transporter (ASBT; 57% suppression, $P = 0.04$) was noted to be lower in HS pigs compared with the CON group and the mRNA level of OSTβ (74.8%, $P < 0.01$) was greater in HS group than PF group. However, no change was observed for genes that are involved in BA transport in the ileum and the cecum of the PF pigs compared with CON pigs.

3.6. Bile acids signaling in intestine and liver

Although the ileum is the major site for BAs reabsorption, no changes were observed in FXR, TGR5 and FGF19 in the HS and PF groups when compared with the CON group (Fig. 4A). In the cecum, the expressions of membrane receptor TGR5 and GLP-1 were lower by 31% ($P = 0.05$) and 45% ($P < 0.01$), respectively, in the HS group, while few changes were observed in the PF group (Fig. 4B).

In the liver (Fig. 4C), remarkable reductions in FXR (24%, $P = 0.02$), TGR5 (42%, $P = 0.002$) and FGFR4 (36%, $P = 0.009$) expressions were observed in HS pigs compared with the CON pigs, and there was no significant difference of SHP or KLβ mRNA expression. Meanwhile, the mRNAs of TGR5 (30%, $P = 0.04$), FGFR4 (38%, $P = 0.002$) and KLβ (43%, $P = 0.0356$) were also suppressed in the PF group.

3.7. Correlations among liver bile acid-related genes

Pearson's correlation (Table 3) revealed strong positive correlations

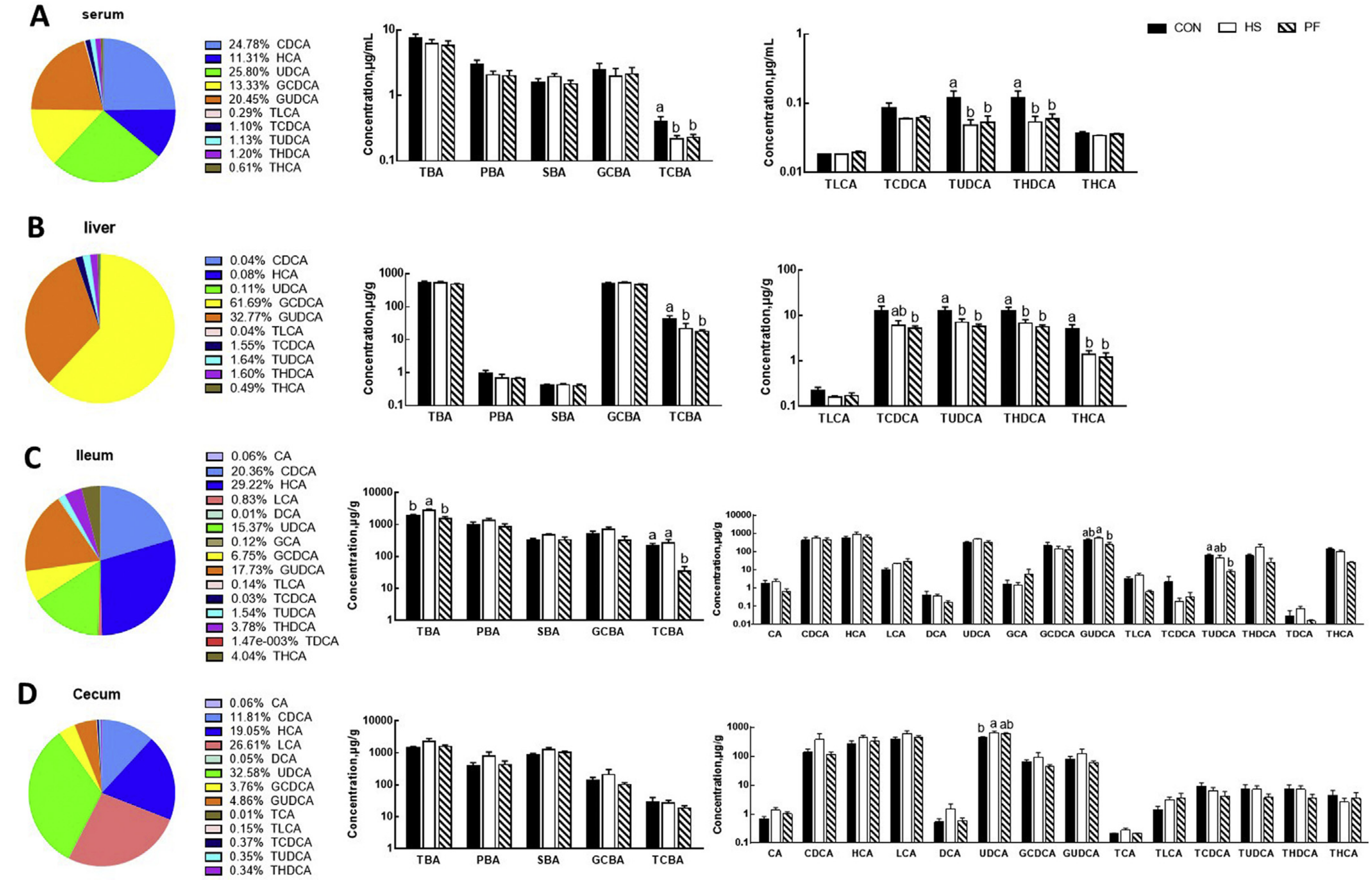

Fig. 1. Alteration of BA profiles in serum (A), liver (B), ileum (C) and cecum (D) after chronic heat exposure. Values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$).

($P < 0.01$) in FXR or TGR5 with FGFR4 (r = 0.5398 and r = 0.6814), CYP27A1 (r = 0.6758 and r = 0.8524), BAAT (r = 0.7980 and r = 0.5405), BACS (r = 0.8190 and r = 0.6745), BSEP (r = 0.5692 and r = 0.6280), OATP1B3 (r = 0.6923 and r = 0.6262) and NTCP (r = 0.7694 and r = 0.6938), respectively. Additionally, TGR5 was positively correlated with KLβ (r = 0.4803, $P = 0.01$).

Significant positive correlations between CYP7A1 and BSEP (r = 0.4199, $P = 0.03$) and NTCP (r = 0.4733, $P = 0.01$) were observed, respectively. In addition, CYP27A1 was positively correlated ($P < 0.01$) with BAAT (r = 0.5274), BACS (r = 0.7529), BSEP (r = 0.6440), OATP1B3 (r = 0.7159) and NTCP (r = 0.8052). However, the correlations between FXR and SHP (r = 0.3610, $P = 0.06$), CYP7A1(r = 0.1542, $P = 0.44$) and CYP8B1(r = 0.1689, $P = 0.40$) or TGR5 and SHP (r = −0.0415, $P = 0.84$), CYP7A1 (r = 0.3473, $P = 0.08$) and CYP8B1 (r = 0.1237, $P = 0.54$) were not significant.

3.8. Instestinal bacteria involved in bile acids biotransformation

Deconjugation and dehydroxylation BAs are fulfilled by intestinal bacteria which possess bile salt hydrolase (Ridlon et al., 2016). Compared with the ileum, increased copy numbers of total bacteria and *Bacteroides* and decreased *Clostridium* was observed in the cecum. However, no differences in the copy numbers of total bacteria, *Bacteroides*, *Clostridium*, *Lactobacillus* and *Enterococcus* were achieved among three groups in the ileum after 21 d heat exposure (Fig. 5A). In the cecum, the total number of bacteria was greater in the PF pigs compared with CON and HS pigs ($P = 0.014$, Fig. 5B).

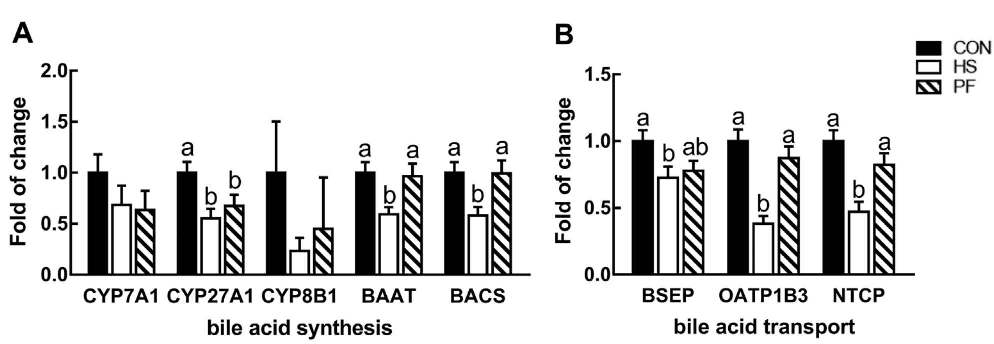

Fig. 2. Relative expression of genes encoding for BA synthesis (A) and transport (B) in liver. Values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$).

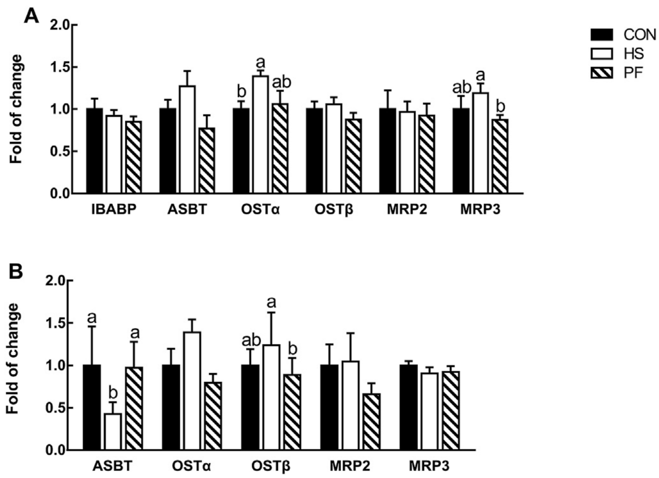

Fig. 3. Expression of BA transporters in ileum (A) and cecum (B) after chronic heat exposure. Values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$).

4. Discussion

Besides growth performance, our recent study and many other studies have found unique regulatory effects in lipid metabolism (Baumgard and Rhoads, 2013; Wen et al., unpublished data). In the current study, we noted that BA metabolism was significantly altered in the pigs exposed to 33 °C for 21 d after a comprehensive investigation of the BA biosynthesis and intestinal recycling. Heat exposure suppressed BA synthesis, conjugation uptake and signaling in the liver and moderately affected ileal BA recycle, which consequently lowered serum TCBAs.

Scattered studies have reported BAs as signature metabolites for heat stress. When pigs were exposed to 12 h-37 °C exposure per day for 7 days, deoxycholic acid (DCA) and hyodeoxycholic acid (HDCA) were increased in the cecal fluid (Wang et al., 2016). Ippolito et al. also observed decreasing BAs, including cholic acid (CA), DCA and all TCBAs, in plasma of rats after 48 h of HS (Ippolito et al., 2014). Most of these studies adopted non-target metabolomics methods, which may not be able to give a full spectrum of BAs. To the best of our knowledge, it is the first study to profile BA composition in compartments important to maintain BA homeostasis after heat stress. Here we noted that liver and serum TCBAs (serum, TUDCA and THDCA; liver, TUDCA, THDCA, TCDCA, THCA) were decreased in the growing pigs exposed to 33 °C for 21 d. TUDCA as a classic endoplasmic reticulum (ER) stress inhibitor, could alleviate ER stress and restore insulin secretion and glucose homeostasis (Guo et al., 2017; Lee et al., 2010; Özcan et al., 2006). Additionally, THDCA induces biliary lipid secretion (Loria et al., 1997), and suggests a cytoprotective factor for hepatocytes (Puglielli et al., 1994). TCBAs are formed with the conjugation of BA and its substrate taurine. In heat-stressed animals, hyperthermia was accompanied by a significant increase of the concentration of taurine, which acted as cryogenic agents to counteract the resulting hyperthermia (Frosini et al., 2002). Taurine posed cytoprotective effects on hepatocytes under the presence of Ca^{2+} (Nakashima et al., 1996), which was due to cytosolic Ca^{2+} leakage that was induced by HS (Roti, 2008). In addition, Cassol et al. reported that taurine is closely related to stress alleviation (Cassol et al., 2010). For instance, a current research indicated taurine supplementation effectively alleviated mitochondrial damage caused by heat exposure (Lu et al., 2018). Reduced TCBAs levels might be used against heat induced ER stress and cell damage.

Because TCBAs can either be synthesized in the liver or re-absorbed from the intestine, it is wondered which route contributes to the reduced TCBAs in the HS pigs. Ileum has the highest expression for BA transporters and FGF19 along the intestinal tract of pigs (Fang et al., 2018), therefore it is the major site for BA re-absorption. Bile acids can be re-absorbed via ASBT-mediated active transport or passive transport. In the ileum, we noted that GUDCA concentration was greater in the ileal digesta in the HS pigs than the CON pigs. The greater GUDCA concentration in the digesta suggests a decreased BA recycling in the ileum of the HS pigs probably due to the overnight fasting, and thus related to the emptying of the digestive tract. Unlike GUDCA, composition of BAs conjugated with taurine as well as their high-affinity receptor ASBT did not show any changes among all treatments. We assume TCBA recycling in the intestine was only moderately modified by heat exposure. In contrast to the lack of changes in the intestine, in the liver, a wide range of BA-related genes were down-regulated in the HS pigs, including critical enzymes for BA synthesis, BA transporters and regulatory signaling. In the process of BA synthesis, CYP7A1 initiates the classical pathway by 7α-hydroxylation of cholesterol and CYP27A1 initiates the alternative pathway. These two pathways produce the majority of PBAs, where the classical pathway may be responsible for 50% or more of the total formation and the alternative pathway may be responsible for 30–50% (Björkhem et al., 2002). CYP8B1 is required for synthesis of CA, maintaining the ratio between CA and chenodeoxycholic acid (CDCA). Under the heat stress condition, as the major contributor to the total formation of BAs, CYP7A1 and CYP8A1 remained unaltered. Accordingly, the level of TBA was maintained in the liver and serum in the HS pigs. However, CYP27A1 in the alternative pathway was down-regulated in the HS pigs, suggesting that the alternative pathway rather than the classical way is affected by heat exposure. In addition to CYP27A1, the two enzymes catalyzing BA conjugation, BAAT and BACS and BAs transporters, NTCP, OATP1B3 and BSEP, were also down-regulated in the HS pigs. Therefore, heat exposure is more likely to suppress alternative BA synthesis, conjugation as well as liver uptake to reduce TCBAs.

Bile acids homeostasis is tightly regulated. The regulatory mechanism is very complex and yet incompletely known. Two major negative feedback regulations have been known for liver BA synthesis. When BAs are recycled from the ileum, ileocytes can sense BAs and secrete FGF19/FGF15, which acts on FGFR4/KLβ in the liver to suppress BA-related genes (Russell, 2009). Although its receptor complex

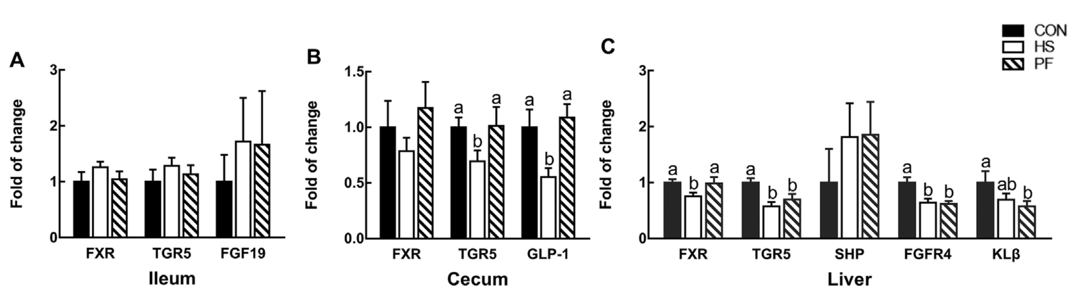

Fig. 4. Expression of BA signaling in ileum (A), cecum (B) and liver (C) after chronic heat exposure. Values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$).

Table 3
Pearson correlation among liver bile acid-related genes.

	TGR5	SHP	FGFR4	KLβ	CYP7A1	CYP27A1	CYP8B1	BAAT	BACS	BSEP	OATP1B3	NTCP
FXR	0.6806*	0.3610	0.5398*	0.3087	0.1542	0.6758*	0.1689	0.7980*	0.8190*	0.5692*	0.6923*	0.7694*
TGR5		−0.0415	0.6814*	0.4803*	0.3473	0.8564*	0.1237	0.5405*	0.6745*	0.6280*	0.6262*	0.6938*
SHP			0.0086	−0.1796	−0.1705	0.0320	0.1385	0.1542	0.2492	−0.0216	−0.0227	0.0625
FGFR4				0.6463*	0.5149*	0.8198*	0.1366	0.4565*	0.5525*	0.6081*	0.5971*	0.7386*
KLβ					0.8106*	0.5275*	0.1476	0.2728	0.3977*	0.3843*	0.2760	0.4884*
CYP7A1						0.3960*	0.0668	0.2269	0.3192	0.4199*	0.3064	0.4733*
CYP27A1							0.1348	0.5274*	0.7529*	0.6440*	0.7159*	0.8052*
CYP8B1								0.2651	0.1993	0.0197	0.1779	0.2285
BAAT									0.6733*	0.6558*	0.7943*	0.8117*
BACS										0.4421*	0.7957*	0.8345*
BSEP											0.6010*	0.6746*
OATP1B3												0.9263*

The values in the table is the correlation coefficient, and the value in * indicates the significance level *P* value ($P < 0.05$).

in the liver, FGFR4/KLβ, was down-regulated in the HS pigs, ileal FGF19 did not vary among treatments. The enterohepatic feedback pathway unlikely contributed to suppress BA synthesis and conjugation in the HS pigs. In addition to the feedback from enterohepatic circulation, BAs in the liver bind to FXR to induce SHP expression, consequently repressing the expression of FXR-target genes, such as CYP7A1, IBABP, BSEP, NTCP and OATP1B3 (Goodwin et al., 2000; Jung et al., 2007; Lu et al., 2000). Altered FXR/retinoid X receptor (RXR) activation in the liver has been reported in heat-stressed dairy cattle (Skibiel et al., 2018b; Zachut et al., 2017). Here we noted a decreased expression of FXR in the HS pigs, but SHP expression did not change accordingly. It should be noted that BAs-induced SHP was very rapid and transient, which was induced by BAs in an acute phase response (Wang et al., 2002). Therefore, the involvement of FXR/SHP in modifying BA-targeted genes after 21-d heat exposure is also excluded. Besides SHP, FXR, as a potent transcription factor, can directly bind to the promoter region of FXR target genes. Using CHIP-seq method, Thomas et al. profiled genome-wide FXR binding activity in the liver and intestine of mouse, showing a strong binding activity towards genes related to BA homeostasis (Thomas et al., 2010). Pearson's correlations also revealed positive correlations between FXR and CYP27A1, BACS, BAAT, NTCP, OATP1B3 and BSEP. The down-regulated expression of genes encoding BA synthesis and conjugation enzymes and BA transporters may be a result of suppressed FXR expression in the HS pigs. Another explanation could be a genome-wide down-regulation in gene transcription in heat-stressed animals (Sørensen et al., 2005). In heat-stressed *C. Elegans*, chaperone production is up-regulated and various metabolic pathways are down-regulated (Wiegant et al., 2009). This down-regulatory effect on gene transcription may be caused by DNA methylations or histone modification. Several studies in pig and cattle have shown that heat exposure leads to changes in DNA methylation (Hao et al., 2016; Skibiel et al., 2018a). In frog, global histone acetylation was noted in the liver during thermal acclimation (Ishihara et al., 2019).

Reduced feed intake during heat stress has always been a concern for farm animals. Although BAs are essential for fat digestion, how feed intake can influence BA homeostasis has not been well studied. Fu and Klaassen found 40% caloric restriction increased the BA pool size by 162% in mice (Fu and Klaassen, 2013). In these mice, increased TDCA and other secondary BAs were correlated with increased expression of CYP7A1 and BACS in the liver (Fu and Klaassen, 2013). Opposite to their results, we noted a 40% feed restriction in growing pigs led to decreased TCBAs in the serum, liver and ileum. Meanwhile, CYP27A1 and BSEP were down-regulated in the liver of the PF pigs. This great discrepancy between the two studies could come from different restriction methods. In our study, we restrictedly fed pigs without balancing other nutrients. In the work of Fu and Klaassen, the mice were on a 60% of normal feed intake but were fed with a nutrient-concentrated diet to ensure sufficient nutrients except energy intake. By comparing the HS to the PF group, we noticed differences in the liver BA-related gene expression despite of a similar pattern in the TCBAs, where FXR, BAAT, BACS, NTCP and OATP1B3 were higher in the PF group than the HS group. Down-regulations in FXR and FXR-target genes in the liver were independent of reduced feed intake in the HS pigs. Therefore, reduced feed intake only partially contributes to the BA homeostasis in the HS pigs.

The majority of BAs in the liver are conjugated with either glycine or taurine. Conjugated BAs are subjected to deconjugation and dehydroxylation by bacteria residing in the large intestine. We have previously shown abundance of *Bacteroides* was positively and *Clostridium*, *Lactobacillus* and *Enterococcus* were negatively correlated with secondary BAs in pigs (Zhang et al., 2018). Here, we confirmed with absolute quantification methods that total bacteria and *Bacteroides* were increased but *Clostridium* was decreased in the cecum compared with the ileum. The transformation from primary BAs into secondary BAs mostly occurred in the cecum and colon (Ridlon et al., 2006; Fang et al., 2018). In our study, the increase of SBA (LCA and UDCA) and decrease of PBA (CDCA and HCA) in the cecum was primarily the result of the conversion of PBA to SBA by *Bacteroides* and *Clostridium,* as already shown by Zhang et al. (2018). In addition, the increase in unconjugated BAs from the ileum to the cecum, together with the high abundance of microbiota in the cecum compared to the ileum, suggests that the bacteria were responsible for the deconjugation of BA, in agreement

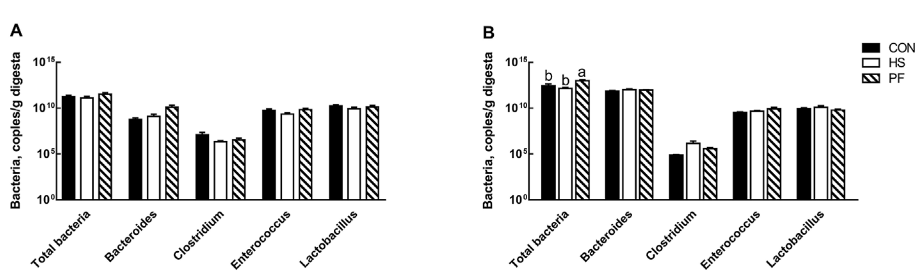

Fig. 5. Bacteria involved in BA biotransformation in ileum (A) and cecum (B). Values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different letter superscripts mean significant difference ($P < 0.05$).

with Masuda (2013). Several publications reporting altered gut microbiome in heat-stressed animals (Contreras-Jodar et al., 2019; Shi et al., 2019). Surprisingly, BA-related bacteria in the ileum and cecum were not affected by 21-d heat exposure or 40% feed restriction despite of a slight increase of total bacteria in the cecum of the PF pigs. The unaltered BA-related bacteria suggested BA-related bacteria community were quite resistant to such treatments. Although cecum is one of the major intestinal compartments for BA metabolism, it is not completely known how cecal BAs contribute to the whole-body BA homeostasis. In the large intestine, TGR5 is mainly located in enteroendocrine L cells. Activation of TGR5 by BAs can stimulate GLP-1 release in the large intestine. Therefore, BAs can regulate postprandial glucose homeostasis (Thomas et al., 2009) and gut motility (Alemi et al., 2013). Our published data have shown that heat exposure increased serum glucose and liver insulin signaling (Xin et al., 2018). In this study, the down-regulated TGR5 in the HS pigs was correlated with the GLP-1 coding gene ($r = 0.53$, $P = 0.005$, data not shown). Altogether, this may indicate that chronic HS affects the production of glucose in the liver to maintain glucose balance, regulated by GLP-1 trough TGR5.

In conclusion, chronic heat exposure can greatly suppress liver activity of synthesis and uptake of TCBAs, which are known as chaperons against ER stress. This could be deleterious to normal function of cells experiencing heat stress. Although TCBAs are decreased in the HS pigs as well as their PF counterparts, the regulatory mechanisms are different. During long-term heat exposure, down-regulated FXR seems to be the trigger for suppression of genes for BA synthesis, conjugation and transport. However, more work needs to be done to validate these hypotheses in the future.

Declaration of interests

The authors declare of no conflict of interest.

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References

Alemi, F., Poole, D.P., Chiu, J., Schoonjans, K., Cattaruzza, F., Grider, J.R., Bunnett, N.W., Corvera, C.U., 2013. The receptor TGR5 mediates the prokinetic actions of intestinal bile acids and is required for normal defecation in mice. Gastroenterology 144, 145–154. https://doi:10.1053/j.gastro.2012.09.055.

Angelin, B., Carlson, L.A., 2010. Bile acids and plasma high density lipoproteins: biliary lipid metabolism in fish eye disease. Eur. J. Clin. Investig. 16, 157–162. https://doi:10.1111/j.1365-2362.1986.tb01323.x.

Baumgard, L.H., Rhoads Jr., R.P., 2013. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1, 311–337. https://doi:10.1146/annurev-animal-031412-103644.

Björkhem, I., Araya, Z., Rudling, M., Angelin, B., Einarsson, C., Wikvall, K., 2002. Differences in the regulation of the classical and the alternative pathway for bile acid synthesis in human liver NO coordinate regulation OF CYP7A1 and CYP27A1. J. Biol. Chem. 277, 26804–26807. https://doi: 10.1074/jbc.M202343200.

Cassol, O.J., Rezin, G.T., Petronilho, F.C., Scaini, G., Gonçalves, C.L., Ferreira, G.K., Roesler, R., Schwartsmann, G., Dal-Pizzol, F., Streck, E.L., 2010. Effects of N-acetylcysteine/deferoxamine, taurine and RC-3095 on respiratory chain complexes and creatine kinase activities in rat brain after sepsis. Neurochem. Res. 35, 515–521. https://doi:10.1007/s11064-009-0089-3.

Chiang, J., 1998. Regulation of bile acid synthesis. Front. Biosci. 3, d176–d193.

Cohen, D.E., 2008. Balancing cholesterol synthesis and absorption in the gastrointestinal tract. J. Clin. lipidol. 2, S1–S3. https://doi:10.1016/j.jacl.2008.01.004.

Contreras-Jodar, A., Nayan, N.H., Hamzaoui, S., Caja, G., Salama, A.A., 2019. Heat stress modifies the lactational performances and the urinary metabolomic profile related to gastrointestinal microbiota of dairy goats. PLoS One 14, e0202457. https://doi: 10.1371/journal.pone.0202457.

Dawson, P.A., Karpen, S.J., 2015. Intestinal transport and metabolism of bile acids. J. Lipid Res. 56, 1085–1099. https://doi: 10.1194/jlr.R054114.

Fang, W., Zhang, L., Meng, Q., Wu, W., Lee, Y.K., Xie, J., Zhang, H., 2018. Effects of dietary pectin on the profile and transport of intestinal bile acids in young pigs. J. Anim. Sci. 96, 4743–4754. https://doi: 10.1093/jas/sky327.

Frosini, M., Sesti, C., Palmi, M., Valoti, M., Fusi, F., Mantovani, P., Bianchi, L., Corte, L.D., Sgaragli, G., 2002. The Possible Role of Taurine and Gaba as Endogenous Cryogens in the Rabbit. Springer US, pp. 335–344.

Fu, Z.D., Klaassen, C.D., 2013. Increased bile acids in enterohepatic circulation by short-term calorie restriction in male mice. Toxicol. Appl. Pharmacol. 273, 680–690. https://doi: 10.1016/j.taap.2013.10.020.

Gabler, N.K., Koltes, D., Schaumberger, S., Murugesan, G.R., Reisinger, N., 2018. Diurnal heat stress reduces pig intestinal integrity and increases endotoxin translocation. Transl. Anim. Sci. 2, 1–10. https://doi: 10.1093/tas/txx003.

Goodwin, B., Jones, S.A., Price, R.R., Watson, M.A., McKee, D.D., Moore, L.B., Galardi, C., Wilson, J.G., Lewis, M.C., Roth, M.E., 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol. Cell 6, 517–526. https://doi: 10.1016/S1097-2765(00)00051-4.

Guo, Q., Xu, L., Liu, J., Li, H., Sun, H., Wu, S., Zhou, B., 2017. Fibroblast growth factor 21 reverses suppression of adiponectin expression via inhibiting endoplasmic reticulum stress in adipose tissue of obese mice. Exp. Biol. Med. 242, 441–447. https://doi: 10.1177/1535370216677354.

Gutzwiller, J., Göke, B., Drewe, J., Hildebrand, P., Ketterer, S., Handschin, D., Winterhalder, R., Conen, D., Beglinger, C., 1999. Glucagon-like peptide-1: a potent regulator of food intake in humans. Gut 44, 81–86. https://doi: 10.1136/gut.44.1.81.

Hao, Y., Cui, Y., Gu, X., 2016. Genome-wide DNA methylation profiles changes associated with constant heat stress in pigs as measured by bisulfite sequencing. Sci. Rep. 6, 27507. https://doi: 10.1038/srep27507.

Holt, J.A., Luo, G., Billin, A.N., Bisi, J., McNeill, Y.Y., Kozarsky, K.F., Donahee, M., Mansfield, T.A., Kliewer, S.A., Goodwin, B., 2003. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. Genes Dev. 17, 1581–1591. https://doi: 10.1101/gad.1083503.

Houten, S.M., Watanabe, M., Auwerx, J., 2006. Endocrine functions of bile acids. EMBO J. 25, 1419–1425. https://doi: 10.1038/sj.emboj.7601049.

Ippolito, D.L., Lewis, J.A., Yu, C., Leon, L.R., Stallings, J.D., 2014. Alteration in circulating metabolites during and after heat stress in the conscious rat: potential biomarkers of exposure and organ-specific injury. BMC Physiol. 14, 14. https://doi: 10.1186/s12899-014-0014-0.

Ishihara, A., Sapon, M.A., Yamauchi, K., 2019. Seasonal acclimatization and thermal acclimation induce global histone epigenetic changes in liver of bullfrog (Lithobates catesbeianus) tadpole. Comp. Biochem. Physiol. Mol. Integr. Physiol. 230, 39–48. https://doi: 10.1016/j.cbpa.2018.12.014.

Jung, D., Elferink, M.G., Stellaard, F., Groothuis, G.M., 2007. Analysis of bile acid--induced regulation of FXR target genes in human liver slices. Liver Int. 27, 137–144. https://doi: 10.1111/j.1478-3231.2006.01393.x.

Katsuma, S., Hirasawa, A., Tsujimoto, G., 2005. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem. Bioph. Res. Co. 329, 386–390. https://doi: 10.1016/j.bbrc.2005.01.139.

Kortner, T.M., Gu, J., Krogdahl, Å., Bakke, A.M., 2013. Transcriptional regulation of cholesterol and bile acid metabolism after dietary soyabean meal treatment in Atlantic salmon (Salmo salar L.). Br. J. Nutr. 109, 593–604. https://doi: 10.1017/S0007114512002024.

Lee, Y.Y., Hong, S.H., Lee, Y.J., Chung, S.S., Jung, H.S., Park, S.G., Park, K.S., 2010. Tauroursodeoxycholate (TUDCA), chemical chaperone, enhances function of islets by reducing ER stress. BIOCHEM BIOPH RES CO Biochem. Bioph. Res. Co. 397, 735–739. https://doi: 10.1016/j.bbrc.2010.06.022.

Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., Staels, B., 2009. Role of bile acids and bile acid receptors in metabolic regulation. Physiol. Rev. 89, 147–191. https://doi: 10.1152/physrev.00010.2008.

Liu, F., Cottrell, J.J., Furness, J.B., Rivera, L.R., Kelly, F.W., Wijesiriwardana, U., Pustovit, R.V., Fothergill, L.J., Bravo, D.M., Celi, P., 2016. Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat--stressed pigs. Exp. Physiol. 101, 801–810. https://doi: 10.1113/EP085746.

Loria, P., Bozzoli, M., Concari, M., Guicciardi, M.E., Carubbi, F., Bertolotti, M., Piani, D., Nistri, A., Angelico, M., Romani, M., 1997. Effect of taurohyodeoxycholic acid on biliary lipid secretion in humans. Hepatology 25, 1306–1314. https://doi: 10.1002/hep.510250601.

Lu, T.T., Makishima, M., Repa, J.J., Schoonjans, K., Kerr, T.A., Auwerx, J., Mangelsdorf, D.J., 2000. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol. Cell 6, 507–515. https://doi: 10.1016/S1097-2765(00)00050-2.

Lu, Z., He, X., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., Gao, F., 2018. Dietary taurine supplementation improves breast meat quality in chronic heat-stressed broilers via activating the Nrf2 pathway and protecting mitochondria from oxidative attack. J. Sci. Food Agric. 99, 1066–1072. https://doi: 10.1002/jsfa.9273.

Masuda, N., 2013. Deconjugation of bile salts by Bacteroids and Clostridium. Microbiol. Immunol. 25, 1–11.

Midtvedt, T., 1974. Microbial bile acid transformation. Am. J. Clin. Nutr. 27, 1341–1347. https://doi: 10.1093/ajcn/27.11.1341.

Nakashima, T., Shima, T., Mitsuyoshi, H., Inaba, K., Matsumoto, N., Sakamoto, Y., Kashima, K., 1996. Taurine in the liver. The function of taurine conjugated with bile acids. Adv. Exp. Med. Biol. 403, 85–92.

Özcan, U., Yilmaz, E., Özcan, L., Furuhashi, M., Vaillancourt, E., Smith, R.O., Görgün, C.Z., Hotamisligil, G.S., 2006. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 313, 1137–1140. https://doi: 10.1126/science.1128294 https://doi.org/10.1111/j.1348-0421.1981.tb00001.x.

Pearce, S., Mani, V., Weber, T., Rhoads, R., Patience, J., Baumgard, L., Gabler, N., 2013a.

Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J. Anim. Sci. 91, 5183–5193. https://doi: 10.2527/jas.2013-6759.
Pearce, S.C., Gabler, N.K., Ross, J.W., Escobar, J., Patience, J.F., Rhoads, R.P., Baumgard, L.H., 2013b. The effects of heat stress and plane of nutrition on metabolism in growing pigs. J. Anim. Sci. 91, 2108–2118. https://doi: 10.2527/jas.2012-5738.
Pollmann, D., 2010. Seasonal effects on sow herds: industry experience and management strategies. J. Anim. Sci. 88 (Suppl. 3), 9 (Abstr.).
Puglielli, L., Amigo, L., Arrese, M., Núñez, L., Rigotti, A., Garrido, J., González, S., Mingrone, G., Greco, A.V., Accatino, L., 1994. Protective role of biliary cholesterol and phospholipid lamellae against bile acid-induced cell damage. Gastroenterology 107, 244–254. https://doi: 10.1016/0016-5085(94)90083-3.
Qu, H., Donkin, S.S., Ajuwon, K.M., 2015. Heat stress enhances adipogenic differentiation of subcutaneous fat depot-derived porcine stromovascular cells. J. Anim. Sci. 93, 3832–3842. https://doi: 10.2527/jas.2015-9074.
Ridlon, J.M., Harris, S.C., Bhowmik, S., Kang, D.-J., Hylemon, P.B., 2016. Consequences of bile salt biotransformations by intestinal bacteria. Gut Microb. 7, 22–39. https://doi: 10.1080/19490976.2015.1127483.
Ridlon, J.M., Kang, D.-J., Hylemon, P.B., 2006. Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. 47, 241–259. https://doi: 10.1194/jlr.R500013-JLR200.
Roti, J.L.R., 2008. Cellular responses to hyperthermia (40–46°C): cell killing and molecular events. Int. J. Hyperth. 24, 3–15. https://doi: 10.1080/02656730701769841.
Russell, D.W., 2003. The enzymes, regulation, and genetics of bile acid synthesis. Annu. Rev. Biochem. 72, 137–174. https://doi: 10.1146/annurev.biochem.72.121801.161712.
Russell, D.W., 2009. Fifty years of advances in bile acid synthesis and metabolism. J. Lipid Res. 50 (Suppl. ment), S120–S125. https://doi: 10.1194/jlr.R800026-JLR200.
Russell, D.W., Setchell, K.D., 1992. Bile acid biosynthesis. Biochemistry 31, 4737–4749. https://doi: 10.1021/bi00135a001.
Sanders, S.R., Cole, L.C., Flann, K.L., Baumgard, L.H., Rhoads, R.P., 2009. Effects of acute heat stress on skeletal muscle gene expression associated with energy metabolism in rats. FASEB J. 23 (Suppl. 1) (Abstr.).
Sanz Fernandez, M., Stoakes, S.K., Abuajamieh, M., Seibert, J.T., Johnson, J.S., Horst, E.A., Rhoads, R.P., Baumgard, L.H., 2015. Heat stress increases insulin sensitivity in pigs. Phys. Rep. 3, e12478. https://doi: 10.14814/phy2.12478.
Shi, D., Bai, L., Qu, Q., Zhou, S., Yang, M., Guo, S., Li, Q., Liu, C., 2019. Impact of gut microbiota sturcture in heat-stressed broilers. Poult. Sci. 0, 1–9. https://doi: 10.3382/ps/pez026.
Siperstein, M., Jayko, M., Chaikoff, I., Dauben, W., 1952. Nature of the metabolic products of C14-cholesterol excreted in bile and feces. Exp. Biol. Med. 81, 720–724. https://doi:10.3181/00379727-81-19999.
Skibiel, A., Peñagaricano, F., Amorín, R., Ahmed, B., Dahl, G., Laporta, J., 2018a. In utero heat stress alters the offspring epigenome. Sci. Rep. 8, 14609. https://doi.org/10.1038/s41598-018-32975-1.
Skibiel, A.L., Zachut, M., do Amaral, B.C., Levin, Y., Dahl, G.E., 2018b. Liver proteomic analysis of postpartum Holstein cows exposed to heat stress or cooling conditions during the dry period. J. Dairy Sci. 101, 705–716. https://doi: 10.3168/jds.2017-13258.
Sørensen, J.G., Nielsen, M.M., Kruhøffer, M., Justesen, J., Loeschcke, V., 2005. Full genome gene expression analysis of the heat stress response in Drosophila melanogaster. Cell Stress Chaperones 10, 312–328. https://doi: 10.1379/CSC-128R1.1.
Thomas, A.M., Hart, S.N., Kong, B., Fang, J., Zhong, X. b., Guo, G.L., 2010. Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51, 1410–1419. https://doi: 10.1002/hep.23450.
Thomas, C., Gioiello, A., Noriega, L., Strehle, A., Oury, J., Rizzo, G., Macchiarulo, A., Yamamoto, H., Mataki, C., Pruzanski, M., 2009. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metabol. 10, 167–177. https://doi: 10.1016/j.cmet.2009.08.001.
Torlińska, T., Banach, R., Paluszak, J., Gryczkadziadecka, A., 1987. Hyperthermia effect on lipolytic processes in rat blood and adipose tissue. Acta Physiol. Pol. 38, 361–366.
Wang, L., Lee, Y.K., Bundman, D., Han, Y., Thevananther, S., Kim, C.S., Chua, S.S., Wei, P., Heyman, R.A., Karin, M., 2002. Redundant pathways for negative feedback regulation of bile acid production. Dev. Cell 2, 721–731. https://doi: 10.1016/S1534-5807(02)00187-9.
Wang, L., Urriola, P.E., Luo, Z. h., Rambo, Z.J., Wilson, M.E., Torrison, J.L., Shurson, G.C., Chen, C., 2016. Metabolomics revealed diurnal heat stress and zinc supplementation-induced changes in amino acid, lipid, and microbial metabolism. Phys. Rep. 4, e12676. https://doi: 10.14814/phy2.12676.
Wiegant, F., Surinova, S., Ytsma, E., Langelaar-Makkinje, M., Wikman, G., Post, J., 2009. Plant adaptogens increase lifespan and stress resistance in C. elegans. Biogerontology 10 (1), 27–42. https://doi:10.1007/s10522-008-9151-9.
Xin, H., Zhang, X., Sun, D., Zhang, C., Hao, Y., Gu, X., 2018. Chronic heat stress increases insulin-like growth factor-1 (IGF-1) but does not affect IGF-binding proteins in growing pigs. J. Therm. Biol. 77, 122–130. https://doi: 10.1016/j.jtherbio.2018.08.008.
Yang, T., Shu, T., Liu, G., Mei, H., Zhu, X., Huang, X., Zhang, L., Jiang, Z., 2017. Quantitative profiling of 19 bile acids in rat plasma, liver, bile and different intestinal section contents to investigate bile acid homeostasis and the application of temporal variation of endogenous bile acids. J. Steroid Biochem. 172, 69–78. https://doi.org/10.1016/j.jsbmb.2017.05.015.
Zachut, M., Kra, G., Livshitz, L., Portnick, Y., Yakoby, S., Friedlander, G., Levin, Y., 2017. Seasonal heat stress affects adipose tissue proteome toward enrichment of the Nrf2-mediated oxidative stress response in late-pregnant dairy cows. J. Proteomics. 158, 52–61. https://doi: 10.1016/j.jprot.2017.02.011.
Zhang, L., Wu, W., Lee, Y.-K., Xie, J., Zhang, H., 2018. Spatial heterogeneity and co-occurrence of mucosal and luminal microbiome across swine intestinal tract. Front. Microbiol. 9, 48. https://doi: 10.3389/fmicb.2018.00048.
Zhu, R., Ou, Z., Ruan, X., Gong, J., 2012. Role of liver X receptors in cholesterol efflux and inflammatory signaling. Mol. Med. Rep. 5, 895–900. https://doi: 10.3892/mmr.2012.758.