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Original research article

Comparisons of blood biochemical parameters, digestive enzyme activities and volatile fatty acid profile between Meishan and Yorkshire piglets

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ABSTRACT

This study was conducted to compare physiological characteristics between Meishan and Yorkshire piglets in their early lives. Six healthy purebred Meishan sows and Yorkshire sows with close farrowing dates were used in this research. The piglets sucked their respective sow's milk for 14 days, then they were slaughtered to collect samples of blood, pancreas, contents of stomach, jejunum, cecum, colon as well as feces for analysis of blood biochemical parameters, digestive enzymes, and volatile fatty acid (VFA). The results showed that Yorkshire piglets had higher concentrations of high-density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) ($P < 0.05$). Gastric lipase activity was higher in Meishan piglets but Yorkshire piglets had higher lactase activity ($P < 0.05$). The total VFA together with acetate and propionate in cecum and colon were higher in Meishan piglets than in Yorkshire piglets ($P < 0.05$), but acetate in jejunum and ratio of acetate to propionate in colon were lower in Meishan piglets than in Yorkshire piglets ($P < 0.05$). In conclusion, in early suckling period, significant differences exist in host metabolism and intestinal microbial metabolism between Meishan and Yorkshire piglets.

1. Introduction

Different pig breeds, especially obese and lean breeds, have been proved to be different in physiological and biochemical characteristics. He et al. (2012) analyzed the blood metabolites of obese Ningxiang pigs (a Chinese indigenous breed) and lean Duroc × Landrace × Yorkshire crossbred pigs after weaning using nuclear magnetic resonance-based metabolomics. The result indicated that significant differences existed in lipids synthesis, lipids oxidation, energy metabolism and amino acids metabolism between the two type pigs. Our previous study on the fecal microbial community of Meishan and Yorkshire piglets showed that Meishan piglets had a stronger ability in volatile fatty acid (VFA) production, and Yang et al. (2014) found that more bacteria belonged to the Firmicutes phyla. Both studies suggest that there are discrepancies in metabolic characteristics between obese and lean piglets after weaning.

To date, only a few studies have focused on physiological characteristics in suckling period without solid feed intake. Kelly et al. (1991) showed that genetically identical piglets fed maternal milk from different breed sows displayed differences in lactase activity, villus height and crypt depth. It was also reported that genetically different piglets fed the same sow milk presented little variance in body weight gain in the late period (Rzasa et al., 2002). These results indicated that maternal milk and genetics affected piglet development in the suckling period. But there is no study regarding the differences on the physiological and biochemical characteristics between obese and lean piglets in early suckling period to our knowledge.

Based on these studies, we hypothesized that the early physiological and biochemical characteristics of obese Meishan (a Chinese indigenous breed) piglets and lean Yorkshire piglets were different.

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Thus, blood biochemical parameters, digestive enzyme activities and VFA concentrations of the two breeds were determined during suckling period.

2. Materials and methods

2.1. Animal management and experimental design

Six healthy purebred Meishan sows and Yorkshire sows with close farrowing date were recruited in this research. All of the sows had 3 to 4 times of accouchement. The sows were given the same type and amount of diets based on corn and soybean. The contents of corn, soybean, fish meal, wheat bran and premix were 63, 24, 3, 6 and 4%, respectively. The nutrient levels of crud protein, ether extracts and crude fiber were 20.67, 3.22 and 3.21%, respectively. The sows were reared individually in one barn with nursing pens, the conditions were identical. After delivery, all piglets were reared on nursing pens until the end of the experiment.

On day 14 postnatal, 1 piglet was randomly selected from each litter (a total of 12 piglets), weighed, and then slaughtered. Before slaughter, blood samples were collected from the vena cava and centrifuged at 1,400 × *g* for 10 min at 4°C. Serum samples were collected and stored at −20°C for future analysis.

2.2. Digesta and feces collection

Digesta samples and feces were collected from stomach, anterior jejunum, cecum and colon, and then stored at −80°C for digestive enzymes and VFA analysis.

2.3. Blood biochemical parameters

Total protein (TP), albumin (ALB), globulin (GLO), glucose (GLU), urea, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) of serum were determined by automatic biochemistry analyzer (AU-400, Olympus, Japan) with kits according to manufacturer's instruction.

2.4. Digestive enzyme activities

Contents of stomach, jejunum and pancreas were prepared for digestive enzymes determination by mixing 1 g contents with 3 mL deionized water. Through centrifugation (2,500 × *g* for 10 min at 4°C), supernatant fluids were obtained to determine activities of amylase, lipase and protease by using colorimetric method according to the instruction of the manufacture (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Volatile fatty acid concentrations

The determination of VFA concentrations was described as previous study (Qin, 1982). Briefly, 0.3-g fecal samples were put in tubes, then 1 mL deionized water was added to each tube. After centrifugation (9,000 × *g* for 10 min at 4°C), 500 μL supernatant fluids were obtained and 0.1 mL 25% (wt/vol) metaphosphoric acid were added to them. The mixtures were injected into gas chromatograph (GC-14B, Shimadzu, Japan) for VFA determination. Parameter settings of the instrument were as follows, temperatures of capillary pipette column, vaporization and detection were 130, 180, and 180°C, respectively. The pressures of nitrogen (the carrier gas), hydrogen and oxygen were 60, 50 and 50 kPa, respectively.

2.6. Statistical analysis

All the experimental data are shown as means ± SEM. Data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL). Significant level was determined by *T*-test. We considered $P < 0.05$ as statistical significance.

3. Results

3.1. Comparisons of blood biochemical parameters between Meishan and Yorkshire piglets

Blood biochemical parameters measured in the current experiment are presented in Table 1. The concentrations of HDL-C and TC were significantly lower in Meishan piglets than in Yorkshire piglets ($P < 0.05$). Meishan piglets also had lower concentrations of TG and LDL-C, although this difference did not reach statistical significance. Other parameters were not significantly affected by breed.

3.2. Comparisons of digestive enzyme activities between Meishan and Yorkshire piglets

Meishan piglets had higher activity of gastric lipase but lower activity of jejunal lactase than Yorkshire piglets ($P < 0.05$) (Table 2). Activity of jejunal amylase in Meishan piglets trended to be lower than that in Yorkshire piglets ($P = 0.09$). There was no valid data of pepsin and jejunal protease activities, which were under detection level of the enzyme kits.

3.3. Comparisons of VFA concentrations between Meishan and Yorkshire piglets

Concentrations of acetate, propionate and total VFA in the cecum contents of Meishan piglets were significantly higher than those of Yorkshire piglets, while acetate concentration in jejunum content was lower in Meishan piglets than in Yorkshire piglets ($P < 0.05$) (Table 3). Molar percentages of acetate, propionate and butyrate in the cecum, colon and feces samples of the two breeds are presented in Table 4. Compared with Yorkshire piglets, Meishan piglets had a higher proportion of propionate in the cecum and colon, but a lower acetate:propionate ratio in the colon ($P < 0.05$). In jejunum, no VFA were detected except for acetate because of their extremely low concentrations.

Table 1
Blood biochemical parameters of Meishan and Yorkshire piglets.

Item	Meishan piglets	Yorkshire piglets	*P*-value
TP, g/L	58.20 ± 6.19	61.38 ± 4.61	0.689
ALB, g/L	37.38 ± 3.71	36.80 ± 1.86	0.891
GLO, g/L	20.82 ± 3.18	23.75 ± 3.01	0.518
GLU, mmol/L	7.70 ± 0.74	8.93 ± 0.58	0.218
Urea, mmol/L	4.52 ± 0.87	3.65 ± 0.19	0.351
TG, mmol/L	0.91 ± 0.18	1.49 ± 0.30	0.129
HDL-C, mmol/L	1.53 ± 0.09^{b}	2.28 ± 0.27^{a}	0.036
LDL-C, mmol/L	2.14 ± 0.35	3.15 ± 0.58	0.166
TC, mmol/L	3.57 ± 0.33^{b}	5.45 ± 0.62^{a}	0.023

TP = total protein; ALB = albumin; GLO = globulin; GLU = glucose; TG = triglycerides; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol.
^{a,b} Within a row, means without a common letter differ ($P < 0.05$).

Table 2
Digestive enzyme activities of Meishan and Yorkshire piglets.

Item	Gut segment	Enzyme activity, U/mg prot		*P*-value
		Meishan piglets	Yorkshire piglets	
Lipase	Stomach	3.01 ± 0.56[a]	1.54 ± 0.08[b]	0.039
	Jejunum	0.47 ± 0.97	0.40 ± 0.09	0.585
	Pancreas	4.26 ± 0.67	3.33 ± 0.52	0.294
Amylase	Stomach	1.06 ± 0.20	0.85 ± 0.21	0.481
	Jejunum	12.50 ± 1.71	18.10 ± 2.44	0.094
	Pancreas	254.83 ± 57.49	178.24 ± 19.35	0.175
Protease	Pancreas	705.50 ± 77.56	780.90 ± 93.83	0.585
Lactase	Jejunum	329.21 ± 27.25[b]	580.03 ± 62.31[a]	0.005

[a,b] Within a row, means without a common letter differ ($P < 0.05$).

Table 3
Concentrations of VFA in different gut segments of Meishan and Yorkshire piglets.

Item	Gut segment	VFA concentration, mmol/L		*P*-value
		Meishan piglets	Yorkshire piglets	
Acetate	Jejunum	3.93 ± 0.46	6.12 ± 0.99	0.046
	Caecum	272.91 ± 23.77[a]	227.97 ± 16.16[b]	<0.001
	Colon	119.97 ± 16.75	138.56 ± 19.92	0.504
	Feces	19.89 ± 2.54	14.95 ± 4.03	0.303
Propionate	Caecum	100.89 ± 38.52[a]	72.23 ± 8.50[b]	0.001
	Colon	55.43 ± 12.05	40.70 ± 10.37	0.376
	Feces	9.24 ± 1.61	5.22 ± 1.71	0.136
Butyrate	Caecum	46.79 ± 17.03	48.25 ± 10.80	0.942
	Colon	29.86 ± 9.11	26.62 ± 10.31	0.823
	Feces	5.40 ± 1.20	4.74 ± 2.01	0.169
TVFA	Caecum	454.09 ± 37.14[a]	387.72 ± 28.71[b]	0.002
	Colon	229.75 ± 43.96	229.85 ± 44.70	0.999
	Feces	40.60 ± 4.90	28.93 ± 8.01	0.220
BCFA	Caecum	20.09 ± 1.87	25.24 ± 3.44	0.353
	Colon	15.09 ± 4.58	14.77 ± 4.12	0.959
	Feces	3.90 ± 0.72	2.54 ± 0.73	0.240

TVFA = total volatile fatty acid; BCFA = branched chain fatty acid.
[a,b] Within a row, means without a common letter differ ($P < 0.05$).

Table 4
Molar percentages of acetate, propionate and butyrate in Meishan and Yorkshire piglets.

Item	Gut segment	Molar percentage of VFA		*P*-value
		Meishan piglets	Yorkshire piglets	
Acetate:TVFA	Caecum	0.63 ± 0.01	0.6 ± 0.04	0.308
	Colon	0.54 ± 0.03	0.65 ± 0.05	0.134
	Feces	0.5 ± 0.04	0.54 ± 0.08	0.610
Propionate:TVFA	Caecum	0.23 ± 0.02[a]	0.18 ± 0.01[b]	0.041
	Colon	0.24 ± 0.01[a]	0.17 ± 0.01[b]	0.003
	Feces	0.22 ± 0.02	0.17 ± 0.02	0.110
Butyrate:TVFA	Caecum	0.073 ± 0.02	0.12 ± 0.02	0.210
	Colon	0.12 ± 0.02	0.1 ± 0.03	0.492
	Feces	0.13 ± 0.03	0.15 ± 0.04	0.737
Acetate:propionate	Caecum	2.76 ± 0.24	3.36 ± 0.39	0.347
	Colon	2.35 ± 0.24[b]	4.1 ± 0.58[a]	0.029
	Feces	2.38 ± 0.33	3.44 ± 0.82	0.295

TVFA = total volatile fatty acid; Acetate:TVFA = the ratio of acetate to TVFA, etc.
[a,b] Within a row, means without a common letter differ ($P < 0.05$).

4. Discussion

4.1. Blood biochemical parameters reflect the differences of fat metabolism between Meishan and Yorkshire piglets

Blood biochemical parameters can reflect physiological state of the body. In the present study, we observed that Yorkshire piglets had a higher concentration of HDL-C than Meishan piglets, together with the results of LDL-C that no statistical significance existed between the two breeds, indicating that more cholesterol in the serum of Yorkshire piglets could be transported into the liver and be oxidized. But considering the higher serum TC concentration of Yorkshire piglets, we consider this may indicate that Yorkshire piglets absorb more cholesterol from sow's milk or have a higher endogenous synthesis rate. We also found that serum TG was lower in Meishan piglets than in Yorkshire piglets, this result is contrary to the observation from a study that TG concentration was significantly higher in obese Ningxiang pig than in lean crossbred (He et al., 2012), whereas consistent with Pond's research (Pond et al., 1980). The reason may related to the growth period and the diet composition.

Additionally, higher ratio of acetate to propionate in the cecum and colon of Meishan piglets led to an increase of gluconeogenesis (Nicholson et al., 2012), but the present study found concentration of serum GLU was lower in Meishan piglets than in Yorkshire piglets, this result indicated that the increased GLU concentration may be used in glycogen synthesis, glucose oxidation or fatty acid synthesis. And it was reported that increased GLU metabolism can provide more acetyl CoA and ATP for the *de novo* synthesis of fatty acids (Rodgers et al., 2008; Vander Heiden et al., 2009). Therefore, basing on the previous studies, we hypothesized that more GLU were converted to triglycerides for fatty acid synthesis in Meishan piglets, but this hypothesis remains to be confirmed.

4.2. Functions of digestive enzymes on intestinal microbiota metabolism

We observed that gastric lipase activity was significantly higher than jejunal lipase activity in both breeds in suckling period. The result suggested that the stomach developed faster than intestine, and gastric lipase played more important roles than jejunal lipase in lipolysis. A similar result was also found in a previous study (Henning, 1981). In addition, gastric lipase activity was higher in Meishan piglets than in Yorkshire piglets, thus more milk fat could be degraded into fatty acids which were transported into fat tissue or to the liver for triglycerides synthesis and deposited as fat in Meishan piglets. Therefore, higher gastric lipase activity may lead to higher back fat thickness (BFT) and the ratio of back fat thickness (RBFT) in Meishan piglets than in Yorkshire piglets (data were not included).

Lactose can be degraded into GLU and galactose by lactase that is mainly synthesized and secreted by the small intestine epithelial cells (Raul et al., 1978). Glucose and galactose are not only host energy sources, but also promoters in the development of brain and neuron, as well as in the growth performance (Bano, 2013). Different levels of lactose were added into weaning feed in a research, which resulted that high level of lactose could significantly promote growth performance of piglets within a certain range (Kim et al., 2010). In the present study, the two breed piglets were fed similar levels of milk lactose. However, Meishan piglets had lower activities of lactase and amylase than Yorkshire piglets, which might lead to more undigested or unabsorbed lactose, and starch was transported into the large intestine. Increased substances were used to produce more microbial fermentation products by microbiota, such as VFA. Some other studies that focused on VFA production also had similar results (Schwiertz et al., 2010). The reason may be microbiota which promotes VFA production and belongs to Firmicutes phyla, especially *Ruminococcus*, *Clostridia cluster IX* and *Clostridia Cluster XIVa* are relatively higher in obese animals than in lean animals (Tremaroli and Bäckhed, 2012). These differences in the abundance of specific microbiota also existed in Erhualian (obese breed) and Landrace (lean breed) (Luo et al., 2012).

5. Conclusion

In conclusion, in the early suckling period, Meishan piglets had lower concentrations of serum TC and HDL-C, but higher concentrations of acetate, propionate, total VFA and proportions of propionate in cecum and colon, as well as a higher activity of gastric lipase than Yorkshire piglets. These results prove that significant differences exist in the activities of blood biochemical parameters, digestive enzymes and VFA concentrations between Meishan and Yorkshire piglets.

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