Dietary Fiber for Gestating Sows During Heat Stress: Effects on Reproductive Performance and Stress Level

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Research

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Abstract

Background: Heat stress is an important issue in swine farms, which causes economic loss and compromises sows health. The addition of fiber to the diet is an option in modifying intestinal health. This study was designed to evaluate the effect of fiber level on reproductive performance, intestinal microbiota and integrity, and metabolism of gestating sows, and its carry-over effect on the lactation period during heat stress.

Methods: A total of 36 gestating sows (90 day-pregnant) were allotted to three treatments with 12 replicates. The diets included 3% (LF), 4.5% (MF), and 6% (HF) crude fiber. All gestating sows fed 2.5 kg of diet daily and diets contained 3150 kcal/kg of ME, 14% crude protein (CP), and 0.58% standardized ileal digestibility of lysine.

Results: Sows fed the HF diet showed a lower respiratory rate and hair cortisol concentration compared with the LF treatment. The HF diet increased the feed intake of sows compared with the LF diet, while it decreased the farrowing duration. The HF diet increased the constipation index compared with the MF and LF diets. Sows in the HF treatment showed a greater piglet weight and litter weight compared with the LF treatment at weaning. Sows in the LF treatment showed the highest digestibility of CP and the greatest digestibility of acid detergent fiber was observed in sows fed HF diet. The HF diet increased lying and decreased standing, and sham chewing behavior compared with the LF diet. The concentration of acetate and total short-chain fatty acid were increased in sows fed the HF diet. The gene expression of glucose transporter 3 and 4 was increased in the HF treatment. The gene expression of heat shock protein70 was decreased in the HF treatment.

Conclusion: Increasing dietary fiber level decreased stress level and improved farrowing duration and reproductive performance.

Introduction

Over the past two decades, heat stress has become a major concern in the animal production industry due to global warming. Besides global warming, the continuous breeding programs improved sows performance and in turn increased their susceptibility to stressors. Heat stress elicits metabolic, behavioral, and physiological changes including increased respiration, and decreased feed intake at the expense of reduced milk production [1,2]. The reaction of animals to reduce feed intake and metabolic rate is a natural attempt to reduce metabolic heat production [3,4]. The reduction of voluntary feed intake and milk production are primarily responsible for low reproductive performance [2,5]. In addition to the influence on feed consumption, a high ambient temperature may affect the behavior and welfare of heat-stressed lactating sows. The heat stressed-lactating sows consume feed more frequently but rather smaller meals, and spending less time lying compared with not stressed sows [6,7]. The change in diet may directly affect the lying or feeding behavior of sows suffering from heat stress that may significantly affect lactation performance.

Proper protein and energy feeding has been always a challenge to optimize the performance of farm animals during heat stress [5,8,9]. During heat stress, the decrease of dietary fiber and protein has been a logical procedure to encourage feed intake and decrease heat production [10]. On the other hand, a change in feed composition from low-fiber to high-fiber has been associated with improved intestinal integrity [11,12], nutrient digestibility [13] colostrum fat [14,15], fecal score [14,16], and welfare [17]. Dietary fibers are subjected to bacterial fermentation and production of short-chain fatty acid (SCFA), which increases intestinal health [16,18-20]. The improved intestinal integrity during gestation may reflect in the lactation period with greater potential for digestibility of nutrients and possibly more efficient digestion. Particularly that the feed intake of lactating sows is almost two times higher than gestating sows and even a small increase of dietary fiber level may induce further stress and compromise litter performance [21]. It has been reported that fibrous diets increase the frequency of meals, spent time eating, and decreased lying time [9]. The decreased lying time can adversely affect reproductive performance because of the lower accessibility of litter to the mammary gland [7]. During lactation, at even moderately heat stress, reduction of feed intake may be magnified by decreasing milk yield. Therefore, supplementation of fiber can be a risk for lactating sows health and welfare. In turn, during gestation the feed intake is limited and it significantly decreases the heat production during digestion. However, little is known about the amount of dietary fiber and its effect on heat-stressed gestating sows. Regarding the importance of fiber to improve intestinal integrity and increase satiety in gestating sows, it seems necessary to understand how much dietary fiber can be adequate during high ambient temperature. The addition of fat to the diet is another dietary program to maintain adequate energy support during heat stress. Fat includes 2.25 times greater energy density rather than carbohydrate, as well as lower heat increment during the digestion process [5]. In the current study, gestating sows were fed with different fiber concentrations to evaluate the influences of fiber levels on reproductive performance, behavior, digestibility of nutrients, stress level, and concentration of metabolites in the intestine.

Material And Methods

Animals and management

The management protocol was according to Kim et al [22]. In brief, artificial insemination has been performed 2 times after the onset of oestrus, and detection of pregnancy was confirmed at d 30 post-breeding using a Pharvision B-mode ultrasound machine (AV 2100V; Ambisea Tech. Corp, Shenzhen, China). During gestation, all sows were housed in individual gestation stalls (2.05 × 1.08 m) with fully slatted concrete flooring. All sows were moved to farrowing crates (2.14 × 2.15 m) on d 112 of gestation. Each crate had a single feeder, and water was always available through a nipple drinker. The gestation and farrowing room temperatures were shown in Figure 1. Heating pads for piglets were located on either side of the farrowing crates and maintained at 36 °C. Lactating sows had ad libitum access to water via a drinker located in the feed trough in each farrowing crate. The feeders were checked 3 times per day to be refilled when required.

Thirty-six multiparous crossbred sows at d 90 of gestation (Landrace × Yorkshire; average initial body weight, 191.6±21 kg) were selected based on parities and body weight (BW). Sows were divided into two blocks (parity three and four) and distributed evenly between three treatments (12 sows/treatment) on d 90 of gestation. The diets were included 3% (LF), 4.5% (MF), and 6% (HF) crude fiber in the corn-soybean-based diets. All gestation diets lactation diets contained 3150 kcal/kg of ME, 14% crude protein (CP), and 0.58% standardized ileal digestibility (SID) of lysine. All sows fed 2.5 kg of diet daily. In the lactation period, sows fed a diet that contained 3300 kcal/kg of ME, 17.8% CP, and 0.88% SID lysine. Starting from the day after farrowing, the ration was gradually increased by one kg per day until the maximum ration was reached (2 kg + 0.6 kg per piglet) about seven days post-partum. Unconsumed feed was weighed daily to determine actual feed intake. All the sows were fed a common corn-soybean meal-based diet (Table 1) as per NRC [23] recommendation.

Body weight and litter performance

Bodyweight was measured on d 90 and d 112 (pre-farrowing) of gestation and d 24 of lactation (weaning) as followed by Kim et al. [5]. Backfat thickness was measured at d 90 and 112 of gestation, and at d 24 of lactation at the 10th rib using an ultrasonic device (Agroscan A16, France). Changes in backfat thickness of sows during lactation were estimated by calculating the difference between backfat thicknesses at d 112 of gestation and backfat thickness at d 24 of lactation. Standard litter traits such as number born and born alive, BW (kg) at birth and weaning, and numbers weaned were recorded. Feed intake (kg/d) of each sow and weaning-to-oestrus interval (d) were also recorded. The value of average daily gain (ADG) of piglets was calculated by final body weight minus the first body weight divided by weaning date (day) multiplied by the number of weaned piglets.

Nutrient digestibility and diet composition

Chromic oxide (0.25%) was added in each diet from d 104 to 112 of gestation as an inert indigestible indicator to measure the apparent total tract digestibility (ATTD) of nutrients. Fecal samples were harvested from the floor during the last 4 days of gestation to measure the ATTD of dry matter (DM), gross energy (GE), CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF). The samples were mixed within pen and dried in a forced-air drying oven at 60 °C for 72 h, and ground in a Wiley mill (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) using a 1-mm screen and used for chemical analysis. Experimental diets and excreta samples were analyzed in triplicate for DM (Method 930.15), CP (Method 990.03), and ADF (Method 973.18) according to AOAC [24]. Gross energy of diets and feces was measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL), and chromium concentrations were determined with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton [25]. An improved method for chromic oxide determination in feed and feces. The NDF was determined gravimetrically with exposure of samples to neutral detergent, amylase, and sodium sulfite, then filtration of samples on a 1.5-µm glass filter [26].

Metabolomics sample preparation and analysis

The metabolites concentrations were evaluated with GC-MS in sows fecal samples. According to He et al [27], 100 mg fecal sample was transferred to 5-ml centrifuge tubes, mixed with 500 µl distilled water, and was vortexed for 60 s. Then, 1000 µL methanol was added to be an internal quantitative standard and vortexed for 30 s. The ultrasound machine was used to hold samples at 25 °C for 10 min, then the centrifuge process (5,000 r/min; 5 °C; 15 min) was performed after 30 min incubation on ice. All the supernatants were placed in 2 mL centrifuge tubes and dried. Then, the dried samples were mixed with 60 µL of methoxyamine solution in pyridine and vortexed (30 s) to be reacted for 120 min at 37 °C. A 60 µL trifluoroacetamide reagent (containing 1% FMCS) was added for 90 min (37 °C) and centrifuged (5,000 r/min; 5 °C; 15 min). The produced supernatant was moved to a sample bottle to be analyzed by Agilent 7890A/5975C GC-MS (Agilent Technologies, Santa Clara, CA, USA).

Gene expressions

Total RNA of placenta tissue was extracted using Trizol (Invitrogen, Carlsbad, CA, USA), and quantified at an absorbance ratio of 260/280 with the Thermo Scientific™ µDrop™ Plate (Thermo Scientific, Waltham, MA, USA) and Multiscan GO (Thermo Scientific, Waltham, MA, USA). The 500 ng of total RNA was reverse transcribed to complementary DNA (cDNA) using Improm-II Reverse transcription system (Promega, Fitchburg, MA, USA). Polymerase chain reaction (PCR) condition and selection of primers were conducted following the description by Hosseindoust et al. [28] and Gao et al. [29]. Reverse transcription-quantitative real-time PCR (RT-qPCR) was conducted using the Real-Time System (Mx3000P, Stratagen, La Jolla, CA, USA). The RT-qPCR primers including heat shock protein-70 (HSP70), glucose transporter (GLUT) 1, GLUT3, GLUT4, and reference gene (β-actin) were designed (Table 2). The relative mRNA expression levels of β-actin as a housekeeping gene was used for normalizing gene expression. The relative fold change of mRNA was determined using 2^{-ΔΔCT} method.

Short chain fatty acids

On d 112 of gestation, approximately 1 g feces was grabbed, weighed, and diluted with 2 mL of deionized water, then to obtain a supernatant the sample was centrifuged at 10,000 g (4°C) for 20 min. Next, a ratio of 9:1 (sample:25% of metaphosphoric acid solution) was mixed and centrifuged at 3,000 g for 10 min. The supernatant was aspirated with a syringe and filtered through a 0.45 mm filter membrane. Acetate, propionate, butyrate, and total SCFA were evaluated using gas chromatography (YL 6500 GC, Gyeonggi-do, Korea; TRB-G43 capillary column with 30m length and inner diameter of 0.53 mm, and film thickness of 3µm), equipped with a flame ionization detector. Column temperature started with 70°C and increased to 150 °C after 3 min. Injector and detector temperature was 250°C, and each injection volume was 1 µL.

Behaviour

Behavior of gestating sows were detected using Geovision GV-1240 video capture combo card (Geovision, Inc., Irvine, CA, USA) and analyzed using EZViewlog (Geovision, Inc., USA) in real-time, according to Kim et al. [30]. Behaviors were checked and evaluated continuously for 7 (0900–1600) h on

d 105 to 112 of gestation. Behaviors registered included: lying, sitting, standing, drinking, sham chewing, licking, sniffing, bar-biting, position change, and farrowing duration.

Hair cortisol

Hair cortisol determination was performed as described previously [31]. In brief, hair samples were shaved from the forehead of sows at the day of 90 and 110 of gestation. The collected hair samples were preserved in aluminum foil and placed in polypropylene tubes to be dried (HM Hyundai Micro Co., Korea). The samples were washed three times with isopropyl alcohol (5 ml) to remove contaminations, then dried at room temperature ($23 \pm 1^\circ\text{C}$) for 7 days. After drying, cortisol extraction was performed by methanol dilution to be analyzed via ELISA kit according to instructions (Cayman Chemical, Ann Arbor, MI).

Blood glucose and insulin

On day 110 of gestation ear vein catheter was subjected to collect blood samples (10 ml) via catheter from all the selected sows before the morning feeding at 6:00 hr at 30-min intervals for 4:30 hr from 06:00 to 10:30 using a non-anticoagulant disposable tube (Becton Dickinson, Franklin, NJ). Serum samples were separated by centrifugation ($3,000 \times g$ for 15 min at 4°C), then stored at -20°C for blood glucose and insulin analysis using commercial kits for glucose (Fujifilm Corp., Saitama, Japan) and insulin (Endocrine Technologies Inc., New York, CA). An automated chemistry analyzer (Fuji Dri-chem 3500i; Fujifilm Corp.) and ELISA device (Biolog MicroStation system) were used for glucose and insulin determination, respectively.

Statistical analyses

The statistical analysis was performed using the GLM procedure (SAS Inst. Inc. Cary, NC). A multiple comparison test was used to compare the effect of fiber levels. Individual sow was used as experimental unit for analysis of all variables. Probability values of $\leq .05$ were considered significant. For metabolites analysis, the collected raw data was analyzed and the metabolites were detected (<http://srdata.nist.gov/gateway/>) and normalized to [$^{13}\text{C}_2$]-myristic acid and stable isotope IS. The statistical analysis was performed with the SIMCA-P+ version 13.0 software package (Umetrics, Umea, Sweden). The variable importance projection (VIP) values of 1.0 and P-values of 0.05 were considered as metabolites that could be evaluated between three fiber treatments. The impact of heat stress on metabolic pathways and metabolite set enrichment analysis were determined according to online tool (<http://www.metaboanalyst.ca/faces/ModuleView.xhtml>) [27].

Results

Heat stress factors

The results of Figure 2 indicated that sows fed the LF diet showed a higher ($P < 0.05$) respiratory rate at days 98, 100, 102, and 104 compared with the HF treatment. There was no change in rectal temperature

among the treatments. The results of Figure 3 showed that hair cortisol concentration of sows was lower ($P < 0.05$) in the HF treatment compared with the MF, and LF treatments.

Sow performance

The results of Table 3 indicated that dietary fiber level did not affect the BW and backfat thickness at d 90, d 112, and weaning times. The HF diet increased ($P < 0.01$) the feed intake of sows compared with the LF diet, while it decreased ($P < 0.01$) the farrowing duration in an opposite manner. Moreover, dietary supplementation with MF decreased ($P < 0.01$) farrowing duration compared with the LF diet, and increased ($P < 0.01$) farrowing duration compared with the HF treatment. The HF diet increased ($P < 0.01$) the constipation index compared with the MF and LF diets. The MF diet showed a higher ($P < 0.01$) constipation index in sows rather than the LF diet. There was no change in weaning to estrus interval among the treatments.

Litter performance

The results presented in Table 4 showed that the fiber level of diet did not affect the total born and weaned piglets. Sows in the HF treatment showed a greater ($P < 0.01$) piglet weight and litter weight compared with the LF treatment at weaning.

Nutrient digestibility

The results presented in Table 5 showed that the digestibility of DM and GE were not affected by dietary fiber. Sows in the LF treatment showed the highest ($P < 0.01$) digestibility of CP. In addition, a greater ($P < 0.01$) digestibility of CP was observed in sows fed MF diet rather than HF diet. There was no change in digestibility of NDF, however, the greatest ($P < 0.01$) digestibility of ADF was observed in sows fed HF diet. Sows in the MF treatment showed a higher ($P < 0.01$) digestibility of ADF compared with the LF treatment.

Behaviors characteristics

The results presented in Table 6 showed that the HF diet increases the lying ($P < 0.01$) and decreased the standing ($P < 0.05$), and sham chewing ($P < 0.05$) compared with the LF diet, whereas it did not affect the sitting, drinking, licking, bar biting, and position change behaviors.

Short-chain fatty acid content

The results presented in Table 7 showed that the concentration of acetate was increased ($P < 0.05$) in sows fed the HF diet. The HF diet did not affect the propionate and butyrate concentration in the feces. Total SCFA content was increased in the HF diet compared with the LF diet.

Plasma insulin and glucose

The results presented in Figure 4 showed that the blood insulin level was increased at min 90 after the meal in the HF diet compared with the MF and LF treatments. However, the dietary fiber level did not affect the insulin level at other times. The blood glucose level was increased ($P < 0.05$) in the LF treatment at min 60 after the meal but decreased ($P < 0.01$) at min 180 compared with the HF treatment. However, the dietary fiber level did not affect the glucose level in other sampling times.

Gene expression

The results presented in Figure 5 showed that the dietary fiber did not affect the gene expression of GLUT1 in the placenta of sows, however, the gene expression of GLUT3 and GLUT4 were increased ($P < 0.01$) in the HF treatments compared with the MF and LF treatments. The gene expression of HSP70 was decreased ($P < 0.01$) in the HF treatment.

Metabolites

PLS-DA results indicated that there are variations in the metabolites based on the different fiber groups (Figure 6). The VIP > 1 and $P < 0.05$ were applied to identify the compounds effects on the variations. The metabolites, including carbohydrates, fatty acids, amino acids, lipids, and organic acids, were detected in multiple biochemical processes in the feces of the sow. The changes in metabolites are shown in Figure 7. The levels of Alanine, phthalic acid, sulfurous acid, hydrocinnamic, 1-phenyl-1,3-h, tryptophanamide, 17 α -hydroxypregnenolone, 1-Butylamine, oxoglutaric acid, phenylacetic acid, thiamine, V41, 1,2-ethanediamine, oleic acid, eicosanoyl-CoA, quinoline, icosenoyl-CoA, propinol adenylate, 2-pentadecanone, methylhexadecanoic acid, isoquinoline, acrylamide, acetyl-CoA, and tyrosinamide, phenylethylamine were increased in the plasma of sows fed the HF diet. The levels of 2-methylhexacosane, lactate, and thiamine pyrophosphate, an active form of thiamine were increased in the plasma of the MF group. The levels of propionic acid and benzofuran were significantly increased in the LF treatment compared with the HF treatment. Based upon the change in metabolites concentrations, metabolic pathways analysis identified the HF diet mainly influenced the pyruvate metabolism, citrate cycle metabolism, glyoxylate, and dicarboxylate metabolism, and thiamine metabolism compared with the MF treatment (Figure 8a). The comparison between the HF and MF treatments showed the change in pyruvate metabolism (Figure 8b).

Discussion

In mammals, blood cortisol is a common indicator of physiological stress [1]. The pregnant animals under exposure to several stressors including fetus growth, hormonal change, metabolical impact, and immunological change, which increases the blood cortisol level compared with non-pregnant sows [32]. Besides blood cortisol, the respiratory rate also could be considered as a measure of heat stress during high ambient temperature [10]. In the current study, respiratory rate was significantly lower in the HF rather than the LF during d 98 to 104 of pregnancy, illustrate lower stress in the HF treatment. In addition, the lower cortisol level in the HF treatment was also in agreement with the report of Huang et al. [9] who reported reducing blood cortisol as the dietary fiber level increased. However, we did not evaluate blood

cortisol, the hair cortisol can be a much proper indicator of chronic heat stress because of the accumulation of cortisol in the hair shaft in a long term period. Moreover, hair sampling is a non-invasive method compared with blood sampling, particularly during a stressful period. The inclusion of 7.5% crude fiber (CF) in gestating sows diet decreased the cortisol concentration in saliva and stool compared with sows fed 2.5% CF [33]. There is a lack of reports of hair cortisol in gestating sows, but several references are showing that hair or wool cortisol is correlated to chronic stress in cattle [34,35] and sheep [36,37]. The mechanisms underlying the influences of dietary fiber on the stress level of gestating sows have not yet been clarified, however, satiety could be associated with lower cortisol. Therefore, dietary fiber can reduce the stress level of pregnant sows during heat stress.

In this study, the diets showed no effects on body weight and backfat thickness during gestation and lactation. In agreement, several studies reported no improvement in sow weight change when high fiber levels were supplemented in gestation diets [9,38]. Investigations on the influence of diet types during the transition period are limited, although gestating sows may have a different nutrient requirement regarding environmental factors and physiological adjustments. In the current study, the HF diets had relatively higher fiber and oil content compared with the LF diets. It has already been suggested that the fibrous diet is a possible solution to increase sows productivity during the transition period [13,39]. The intestinal activity was increased during the periparturient period when fiber-based diets were fed to gestating sows, thereby the risk of constipation decreased during the hours before farrowing [16,40]. Zhuo et al [14] reported an increase in fecal score by increasing dietary fiber levels in gestating sows. However, these results are mostly achieved during the normal environmental condition and there is still a stereotype to avoid using fiber during heat stress due to its higher heat increment by increasing intestinal movements. However, the result of our study shows that the addition of fiber to the diet is a necessity even during heat stress because of a significantly lower farrowing duration in sows fed the HF diet. Gestating sows are fully cared to have a relatively lower farrowing duration because of their serious effects on piglets survivability [5,6]. Acknowledging the significance of diet type, improved nutritional strategy in the transition period to decrease farrowing duration can be considered as a potential alternative to increase sow productivity during stressful periods. The duration of farrowing is not only important for the sow but also for piglets because of critical physical impact, which can lead to the death of piglets [3]. Prolonged farrowing duration is an exhausting and painful period for sows that impose stress on piglets ultimately the increase of stillbirth number [4]. Therefore, farrowing duration is a determinant factor to evaluate the quality of diet or management. This involves greater survivability of piglets, more live-born piglets during lactation to ensure the capability of piglets in accessing the udder as soon as after birth [41]. However, several factors including genetics, body weight, litter size, constipation index, and parity affect farrowing duration [33,42], it recommended that the farrowing duration should not normally be prolonged over than 4 h [14]. In the current study, the average farrowing duration (4.84 h) was higher than recommended values, possibly because of adverse effects of heat stress. The positive effects of the HF diets on farrowing duration can be because of a higher constipation index. Practically, the feed intake of gestating sows is restricted during the last 2 days before the expected farrowing [21]. In this situation, the probability of occurring constipation increases around farrowing due to low feed intake and fiber intake

[14,43]. The high water holding capacity of fiber increase the water content of feces and resulting eases the defecation process [44]. In agreement, Shang et al. [16] showed that the inclusion of 30% wheat bran or 20% of beet pulp to the gestation diet, increased the softness of feces at farrowing. Moreover, they concluded that the inclusion of 15% wheat bran or 10% of beet pulp increased fecal water content in lactating sows. In addition, it was reported that increasing the content of crude fiber in the diet of the sow from 3.8% to 7% increases water consumption and intestine motility around farrowing and thus decreased the constipation risk [40]. Moreover, a longer farrowing duration would worsen the uterine involution and possibly increase the repeat of insemination during the estrus period [45]. However, the result of the current study did not show any relationship between farrowing duration and weaning to estrus interval. Therefore, including low starch and high fiber in the diet of gestating sows is highly recommended during high ambient temperature.

In the current study, there was no fiber effect on litter size. The use of a bulky diet for a period of two months improved litter growth performance during the first week after farrowing [46]. In addition, the high fiber-based diet during the last 3 weeks of pregnancy increased litter weight gain until day 5 of lactation [40]. The growth and development of mammary mainly occur at the late gestation period from 80 to 115 days [15,47]. During the last third of pregnancy, the secretion of endocrine hormones markedly increases the proliferation of epithelial secretory tissues in the alveoli [15,47]. Therefore the greater litter weight at weaning may be associated with the increase of lobular-alveolar number at late gestation, which possibly increases the milk yield in the lactation period. The treatments did not have any effect on the digestibility of DM, GE, and NDF, however, the digestibility of ADF was improved in the HF treatment. The insoluble fraction of fibers is composed of cellulose and lignin that mainly remained unhydrolyzed in exposure to endogenous enzymes during the digestion process [12,19]. Therefore, the fibrous diets increase the transit time, consequently increase digestion and absorption duration. Interestingly, besides the increase of ADF digestibility in the HF treatment, the CP digestibility was reduced when compared with the LF and MF treated sows. Lowell et al. [13] reported a similar result that high dietary fiber decreased the digestibility of protein and increased NDF and ADF digestibility.

The increase of lying posture and decrease of standing and sham chewing behaviors were correlated with decreased respiratory rate. During the gestation period, lying behavior is an indicator of comfort [7,17] and sham chewing is among the most common stereotypical behavior in sows [9]. In the current study, average lying posture duration was higher and standing duration was lower in sows in the HF treatment, indicating that high fiber and low starch content in the diet could increase comfort during heat stress. One possible reason could be the satiety feeling induced by dietary fiber. A reduction of stereotypic behavior and a decrease of lying time have been shown in gestating sows fed 7.5% CF diet treatment rather than sows fed 2.5% CF diet [33], showing that the stress level can be decreased by high dietary fiber inclusion. Meunier et al. [39] reported that the feed restriction of sows increases stereotypic behavior such as sham chewing. It has been already confirmed that dietary fiber is an important factor in increasing satiety and decreasing feeding motivation during gestation, which leads to reduced stereotypic behaviors [17,33]. However, dietary fiber is recommended in normal conditions and there is a lack of reports on the role of fiber during heat stress, particularly that the dietary fiber may increase the heat

increment during the digestion process [8]. Therefore, the result of this study emphasizes that the inclusion of fiber in the diet is necessary not only in normal conditions but also during heat stress to increase satiety and the welfare of gestating sows.

A low dietary starch supply during late gestation increased total SCFA production, which possibly was due to high fiber content and fermentable carbohydrate as substrates of SCFA production at the distal part of the intestine [16,20]. Acetate, propionate, and butyrate are the main end product of the fermentation process in the large intestine [18,48]. There are several factors including the environment, diet, and gut microbiota to determine the amount of SCFA production [11]. It has been reported that dietary fiber is a more important substrate for SCFA production rather than dietary starch [18]. In the literature, the positive relationship between dietary fiber and fecal SCFA was confirmed [16,33,49]. However, SCFA can be produced in all sections of the gastrointestinal tract, colon, and cecum are the main sites of SCFA production [12]. There is a hypothesis that the dietary fiber can encompass carbohydrates and increase their follow to reach the large intestine and ultimately increase fermentation [50]. The most important effects of SCFA are expected to occur on carbohydrate homeostasis, lipid metabolism, immune system, and gastrointestinal health [12,33]. Short-chain fatty acids bind to specific receptors in the intestine including G-protein-coupled receptor 41 and G-protein-coupled receptor 43 to restrict histone deacetylation and have a positive role in body metabolism [51].

The fiber supplementation and its fermentation in the colon increase SCFA production including acetate, butyrate, and propionate [11,16,20]. Soluble fiber provides substrates for fermentation in the colon, which has been reported to increase SCFAs production instead of protein-based metabolites including ammonia, indole, cresol, phenol compounds, and hydrogen sulfide [19]. Metabolomics profiling of fecal samples showed differentiation of the HF from the LF treatment. The multivariate analyses showed that the HF and LF treatments had the most obvious separations among the treatments. The VIP scores were generated to evaluate the metabolites' contribution among different fiber levels in experimental groups. Twenty-five fecal metabolites were altered in sows fed HF diet. Thiamine metabolism, sulfur metabolism, and citrate cycle metabolism were perturbed in the LF treatment compared to the HF. The results showed that the vitamin B group metabolism was improved in fecal samples of the HF sows. Thiamine pyrophosphate is the phosphorylated form of vitamin B1 with a significant role in nervous system development and maintenance [52]. The activity of glyoxalase system can be compromised when the thiamine concentration decrease [53]. The limitation of thiamine availability reduces the proliferation of essential microbes including *B. thetaiotaomicron*, which belongs to the *Bacteroidetes* phylum and *Bacteroides* genus [54]. In addition, it is important that there is a link between thiamine metabolism and thyroid hormone, a necessary hormone for optimal body metabolism, production [55], which may increase sows performance during heat stress. In the current study, we found that the supplementation of high fiber increased the nutrient absorption and metabolism pathways including pyruvate metabolism, citrate cycle, propionate metabolism, and glyoxylate and dicarboxylate metabolism. These pathways play an important role in energy extraction and provision targeting substrate sensing and carbohydrate substrate transporting. In addition, the level of metabolites involved in stress such as sulfurous acid increased in a high fiber diet, which indicated an improved antioxidant status mainly because sulfurous

acid converts to sulfate to protect cells from oxidative stress [56]. 1-Butylamine colorless liquid with an amine group, which has an ammonia-like odor [57]. The increase of 1-Butylamine in the feces of sows fed a high fiber diet may be associated with lower protein digestibility, resulting in more undigested protein flowed to distal sections of gastro intestinal tract.

In conclusion, the results of the present study show that the inclusion of 6% dietary fiber and 7% fat could decrease the adverse effects of heat stress during the gestation period. Further, the carry-over effects of 6% fiber inclusion during the gestation period reflects in the lactation period by improving reproductive performance and litter growth of lactating sows.

Abbreviations

SCFA: Short-chain fatty acid

BW: Body weight

LF: Low fiber

MF: Mideum fiber

HF: High fiber

SID: Standardized ileal digestibility

ME: Metabolizable energy

CP: Crude protein

ATTD: Apparent total tract digestibility

DM: Dry matter

GE: gross energy

NDF: neutral detergent fiber

ADF: Acid detergent fiber

VIP: Variable importance projection

CF: Crude fiber

HSP: Heat shock protein

GLUT: Glucose transporter

Declarations

Acknowledgements

Not applicable.

Author Contributions

All authors contributed to this work. Conceived and designed the experiments: AH and JSK. Performed the experiments: SMO, SHH, JM. Analyzed the data: AH, SMO, HT. Interpreted the data: JYM and JSK. Wrote the paper: AH, SMO, and JSK.

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Availability of data and material

The datasets used and/or analyzed in the current study are available from the authors on reasonable request.

Ethics approval and consent to participate

The protocol for the experiment was approved and sows and piglets were cared according to the guidelines of the Institutional Animal Care and Use Committee of Kangwon National University (KW-170519-1), Chuncheon, Republic of Korea.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Formula and chemical composition of experimental basal diets (as-fed basis)

Item	Crude fiber level %		
	3	4.5	6
Ingredient (%)			
Corn	68.76	48.89	28.98
Whey powder	4.00	4.00	4.00
SBM dehulled	14.06	7.79	1.32
Soy oil	-	3.76	7.53
Wheat bran	5.83	20.13	34.53
DDGS	4.00	12.00	20.00
Salt	0.50	0.50	0.50
TCP	1.36	1.14	0.92
Limestone	0.86	1.05	1.25
DL-Methionine (98%)	0.02	0.02	0.03
L-Lysine (78.8%)	0.08	0.21	0.34
L-Tryptophan (10%)	0.07	0.11	0.17
L-Threonine (98.5%)	0.11	0.05	0.08
Choline-chloride (50%)	0.10	0.10	0.10
Vitamin premix ¹	0.10	0.10	0.10
Mineral premix ²	0.10	0.10	0.10
Phytase	0.05	0.05	0.05
Total	100.00	100.00	100.00
Cost (won/kg)	324	336	349
Chemical composition (%)			
ME (Kcal/kg)	3,140	3,140	3,140
CP	14.00	14.00	14.00
Ca	0.82	0.82	0.82
Av.P	0.38	0.38	0.38
CF	3.00	4.50	6.00

SID. Lys	0.58	0.58	0.58
SID. Met	0.22	0.22	0.22
SID. Met + Cys	0.42	0.42	0.42
SID. Thr	0.52	0.42	0.42
SID. Trp	0.13	0.13	0.13
¹ Supplied per kilogram of vitamin premix: 12,000,000 IU vitamin A, 2,400,000 IU vitamin D3, 132,000 IU vitamin E, 1,500 mg vitamin K3, 3,000 mg vitamin B1, 11,250 mg vitamin B2, 3,000 mg vitamin B6, 45 mg vitamin B12, 36,000 mg pantothenic acid, 30,000 mg niacin, 600 mg biotin, 4,000 mg folic acid. ² Supplied per kilogram of mineral premix: 80,000 mg Fe, 170 mg Co, 8,500 mg Cu, 25,000 mg Mn, 95,000 mg Zn, 140 mg I, 150 mg Se. *Abbreviation: CF, crude fiber.			

Table 2. Primers used for real-time PCR analyzing

Gene	Nucleotide sequence of primers (5'–3')
GLUT1	F: GCAGGAGATGAAGGAGGAGAGC
	R: ACGAACAGCGACACGACAGT
GLUT3	F: GCCCTGAAAGTCCTCGGTTTCCT
	R: ACACGGCGTTGATGCCAGAGA
GLUT4	F: GGCCATCGTCATTGGCATTC
	R: GTCAGGCGCTTCAGACTCTT
HSP70	F: GCCCTGAATCCGCAGAATA
	R: TCCCCACGGTAGGAAACG
HSP, Heat shock protein; GLUT, glucose transporter	

Table 3. Effect of dietary fiber levels in gestation diets on sows performance

Crude fiber, % feed	3	4.5	6	SEM	<i>p</i> -value
BW, kg					
d 90	192.22	190.44	192.33	1.17	0.775
d 112	206.89	206.11	205.52	0.91	0.832
Weaning	169.44	171.00	169.94	1.05	0.830
Loss during Lactation	37.45	35.11	35.58	0.73	0.376
BF, mm					
d 90	21.05	20.46	20.17	0.24	0.336
d 112	21.35	20.75	20.49	1.03	0.163
Weaning	16.24	16.19	16.14	0.19	0.982
Loss during Lactation	5.11	4.59	4.35	4.68	0.456
ADFI kg/d					
During lactation	4.77 ^b	5.05 ^{ab}	5.25 ^a	0.07	0.008
Farrowing duration, h	5.24 ^a	4.92 ^b	4.45 ^c	0.12	<0.001
Constipation index ¹	1.20 ^c	1.80 ^b	2.63 ^a	0.08	<0.001
Weaning to estrus interval, d	5.25	5.15	5.11	0.19	0.826
¹ A score value ranging from 0 to 5: 0, absence of feces; 1, dry and pellet-shaped; 2, between dry and normal; 3, normal and soft, but firm and well formed; 4, between normal and wet; still formed, but not firm; and 5, very wet feces, unformed and liquid. ^{a,b,c} means with different superscripts in the same row differ significantly (<i>p</i> <0.05). CF, crude fiber; SEM, standard error of means; BW, body weight; BF, backfat thickness; ADFI, average daily feed intake.					

Table 4. Effect of dietary fiber levels in gestation diets on litter performance

Crude fiber, % feed	3	4.5	6	SEM	<i>p</i> -value
Litter size					
Total born	12.70	12.40	12.50	0.15	0.721
Weaned	10.30	10.20	10.50	0.21	0.849
Piglet weight, kg					
At birth	1.29	1.31	1.27	0.04	0.937
At weaning	5.77 ^b	5.89 ^{ab}	5.98 ^a	0.03	0.045
Litter weight, kg					
At birth	14.80	15.07	14.30	0.59	0.874
At weaning	56.99 ^b	59.64 ^{ab}	60.79 ^a	0.65	0.043
^{a,b} means with different superscripts in the same row differ significantly ($p<0.05$). CF, crude fiber; SEM, standard error of means.					

Table 5. Effect of dietary fiber levels in gestation diets on apparent total digestibility (%) of nutrients

Crude fiber, % feed	3	4.5	6	SEM	P-value
DM	88.4	88.63	87.27	0.61	0.682
GE	89.33	88.37	87.5	0.59	0.506
CP	88.87 ^a	85.57 ^b	81.17 ^c	1.16	0.001
NDF	64.07	64.73	65.37	0.58	0.714
ADF	55.27 ^c	58.13 ^b	60.77 ^a	0.85	0.002

^{a-b}Means with different superscripts in the same row differ significantly ($p<0.05$).

Abbreviation: CF, crude fiber; SEM, standard error of means; DM, dry matter, GE, gross energy; NDF, neutral detergent fiber; ADF, acid detergent fiber.

Table 6. Effect of dietary fiber levels in gestation diets on sows behavior characteristics

Crude fiber, % feed	3	4.5	6	SEM	<i>p</i> -value
Lying, %	73.60 ^b	75.19 ^{ab}	79.05 ^a	0.91	0.037
Sitting, %	15.01	15.15	14.52	0.90	0.959
Standing, %	11.39 ^a	9.66 ^a	6.43 ^b	0.71	0.011
Drinking, %	4.70	4.30	3.70	0.49	0.716
Sham chewing, times	11.40 ^a	10.20 ^{ab}	7.20 ^b	0.71	0.038
Licking, sniffing, times	3.20	2.80	2.50	0.46	0.835
Bar-biting, times	5.90	4.50	4.10	0.89	0.252
Position change, times	6.80	5.80	4.20	0.60	0.208
^{a,b} means with different superscripts in the same row differ significantly (<i>p</i> <0.05). CF, crude fiber; SEM, standard error of means.					

Table 7. Effect of dietary fiber levels in gestation diets on fecal SCFAs concentration (d 112)

Crude fiber, % feed	3	4.5	6	SEM	P-value
Acetate	69.56 ^b	71.68 ^{ab}	72.67 ^a	0.53	0.041
Propionate	16.8	17.3	17.9	0.43	0.577
Butyrate	7.52	7.41	7.54	0.06	0.725
Total SCFAs	93.85 ^b	96.40 ^a	98.56 ^a	0.61	0.003
^{a-b} Means with different superscripts in the same row differ significantly (<i>p</i> <0.05).					

Abbreviation: CF, crude fiber; SEM, standard error of means; SCFA, short-chain fatty acids.

Figures

Figure 1

Ambient temperature (blue line) and temperature-humidity index (THI; orange line) during experimental period.

Figure 2

Effect of dietary fiber levels in gestation diets on respiratory rate and rectal temperature of sows from d 90 to 114 under high ambient temperature. Values represent means $\pm$ standard error. Asterisks (*) indicate statistical significance ($p < 0.05$). CF, crude fiber.

Figure 3

Effect of dietary fiber levels in gestation diets on hair cortisol accumulation of sows from d 90 to 112 under high ambient temperature. Values represent means $\pm$ standard error. a,b means with different superscripts on the bar differ significantly ($p < 0.05$). CF, crude fiber.

Figure 4

Effect of dietary fiber levels in gestation diets on plasma insulin and glucose (d 112).

Figure 5

Effect of different fiber levels on relative mRNA expression of glucose transport protein and heat shock protein 70 in placenta of sows.

Figure 6

Partial least squares projection to latent structures and discriminant analysis (PLS-DA) based on the fecal compounds data.

Figure 7

Top 30 Significant compounds. Metabolites accountable for class discrimination with VIP > 1 among fiber treatments.

Figure 8

Metabolome view map of the differential metabolites ($VIP > 1$, $P < 0.05$) identified in the feces of sows fed different CF levels (a: 3.0 % vs 6.0 %; b: 4.5 % vs 6.0 %) during late gestation. The x-axis represents the pathway impact and the y-axis represents the pathway enrichment. The node color is based on its P-value, and the node radius is determined based on the pathway impact values. Larger sizes and darker colors represent higher pathway enrichment and impact values, respectively.