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# Applied Animal Behaviour Science

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# High fiber diet reduces stereotypic behavior of gilts but does not affect offspring performance

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#### ARTICLE INFO

Keywords: animal welfare feeding behavior dietary fiber pregnant gilts prenatal stress

#### ABSTRACT

Pregnant sows are often subjected to food restriction, which can compromise their welfare and performance, as well as the performance of their offspring. High fiber diets (HFD) can mitigate the feeling of hunger and, consequently, improve welfare and performance. The aim of this study was to evaluate the impact of feeding pregnant gilts with high a fiber diet on performance measures of sows. Additionally, the behavior and welfare of the sows were assessed. Twenty-eight pregnant gilts were fed either HFD (N = 16) or low fiber diet (LFD; N = 12). We evaluated behavior, salivary cortisol concentration, performance, and feeding motivation. We found an interaction between treatment and feeding time for duration (P = 0.0041) and frequency (P = 0.0128) of shamchewing stereotypy. Sows that received LFD performed the behavior for longer and more often before feeding than after feeding. These results indicate that HFD was beneficial in reducing stereotypic behavior in sows, prior to feeding time, but did not improve performance measures.

#### 1. Introduction

Pregnant sows kept in commercial conditions are subjected to a restricted dietary allocation of about 50-60% of their ad libitum consumption capacity (Lawrence et al., 1988). Moreover, this study is supported by Read et al. (2020), published three decades later, demonstrating this issue is also a concern for contemporary pig production systems (see review by Jarret and Ashworth, 2018). The aim of this dietary restriction is to maintain an adequate body condition score, avoiding metabolic disorders, productive and reproductive consequences (D'Eath et al., 2009, 2018; de Leeuw et al., 2005; De Leeuw et al., 2004; Read et al., 2020; Zonderland et al., 2004). However, the amount of feed supplied, despite meeting the nutritional requirements for maintenance and reproduction, is insufficient to keep the sows satiated and to satisfy the feed motivation (De Leeuw et al., 2004; Meunier-Salaün et al., 2001). Furthermore, it is rapidly consumed whereas adult sows, in a semi-natural environment can spend 63% of their day trying to obtain feed resources (grazing, rooting, and orienting to stimuli) (Stolba and Wood-Gush, 1989). The hunger experienced by pregnant sows fed with commercial diets during gestation (D'Eath et al.,

2009) increases feed motivation, activity, and development of stereotypic behaviors (De Leeuw et al., 2004; Lawrence and Terlouw, 1993; Meunier-Salaün et al., 2001). Broom (1983) defined stereotypies as behavior that is performed in repetitive movements, without any obvious function, and cannot be considered part of the normal repertoire. Restrict-fed sows can develop a variety of stereotypies, such as licking the empty feeder, biting bars of the crate, sham-chewing, increased activity, increased handling of the drinker, and excessive water intake (Douglas et al., 1998; Jensen, 1980; Lawrence and Terlouw, 1993). These abnormal behaviors before and after feeding can result from the frustration caused by restriction of access to, and manipulation of, feed (Danielsen and Vestergaard, 2001; Meunier-Salaün et al., 2001; Robert et al., 1997). Much of the concern regarding these behaviors stems from the fact that they are indicators of poor welfare (Broom, 1983; Broom and Fraser, 2015; Brouns et al., 1994; D'Eath et al., 2018; Mason, 1991, Meunier-Salaün and Bolhuis, 2015; Tatemoto el al, 2019). Poor welfare can lead to changes in performance, immune function, and behavior (Barnett et al., 1983). Moreover, the sows' diet during gestation can modulate the subsequent behavior and welfare of piglets (Bernardino et al., 2016).

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Appleby and Lawrence (1987) argued that hunger is the most important cause of stress in pregnant sows kept in gestation crates. There are few definitions of hunger in the literature. We defined hunger as a sensation induced by an imbalance of satiety indicators that could be aversive

Some studies demonstrate approaches to minimize the sensation of hunger, which involve the quantitative or qualitative alteration of the diet (D'Eath et al., 2009). The use of dietary fiber for pregnant sows is an efficient tool to reduce stereotypies. Crude fiber can also mitigate symptoms of constipation, a very common discomfort of pregnant sows (Jiang et al., 2019), and address many other issues of concern (see review by Jarret and Ashworth, 2018).

Previous studies have reported behavioral indicators of greater satiety in sows fed with high fiber diets (Brouns et al., 1994; Jiang et al., 2019; Lawrence and Terlouw, 1993; Robert et al., 1997; Sapkota et al., 2016; Stewart et al., 2011; Sun et al., 2015), such as increased lateral lying, less abnormal behavior, and lower feeding motivation. These studies have used different sources of dietary fiber for pigs, for example, sugar beet pulp, alfalfa hay, corn germ meal, and silage (Brouns et al., 1994; Danielsen and Vestergaard, 2001; de Leeuw et al., 2005; De Leeuw et al., 2004; Jiang et al., 2019; Robert et al., 1997; Sapkota et al., 2016; Stewart et al., 2011; Zonderland et al., 2004). Because of this great variety of dietary fiber sources, the research outcomes are also variable and, sometimes, controversial and inconclusive. The choice of different fiber for pigs is attached to the local availability of the source and to the physicochemical properties of the fiber such as fermentability, bulkiness, and viscosity (Souza da Silva et al., 2012). A widely available and potentially valuable source of fiber for pigs is the soybean hull. This has approximately 35% crude fiber of good quality, with approximately 59% of neutral detergent fiber and 41% of acid detergent fiber, presenting a greater quantity of hemicellulose when compared with fiber of citrus pulp and alfalfa hay (NRC, 2012).

In the evaluation of the welfare benefits of a high fiber diet, behavioral observation is an extremely important component. Feed motivation tests can be a useful tool to measure how much a diet can be effective promoting satiety (Day et al., 1997). Furthermore, studies of animals with restricted access to feed indicate that feeding is the most important factor in the organization of hypothalamic-pituitary-adrenal axis rhythm (Dallman et al., 1993). Salivary cortisol measurements, taken in both the morning and in the afternoon, provide information about hypothalamic-pituitary-adrenal (HPA) axis homeostasis in sows (Zanella et al., 1998). Our aim was therefore to investigate the impact of a high fiber diet incorporating soybean hulls on the behavior, salivary cortisol concentration, feed motivation, and performance of pregnant gilts.

# 2. Materials and methods

The experiment was carried out at the pig farm of the University of São Paulo, Campus Fernando Costa, in Pirassununga, Brazil. The research was approved by the Ethics Committee on the Use of Animals (CEUA) at the School of Veterinary Medicine and Animal Science (FMVZ) of the University of São Paulo (USP), under the protocol number 3606300114.

## 2.1. Animals

Twenty-eight pregnant gilts from a group of 36 animals of the Top-Gen Afrodite® genetic line (Granja Araporanga - Juaguariaíva-PR) were included in this study. All gilts were artificially inseminated with pooled semen. They were distributed to the treatments by weight on the day after the first insemination. A mature boar, older than 18 months, was used to perform the estrus identification. The insemination protocol adopted was three inseminations, one at the time of estrus identification and the following at 24 and 36 h after the first insemination. The mean age of the animals at insemination was 291 days (SD 20.18 days) and the

average weight was 184.8 kg (SD 22.63 kg).

#### 2.2. Housing and handling

Gilts were kept in group-housing pens, with nine animals per pen. The pens had individual feeders and the animals had ad libitum access to water. Feeding was preceded by a sound stimulus to minimize the animals' anticipatory response to the presence of humans. The pen was 6.7 m wide by 4.4 m long, totaling 29.48 m<sup>2</sup> (3.27 m<sup>2</sup> per animal), disregarding the area of the feeders. The pen had nine individual feeding crates, constructed of masonry, measuring 1.8 m length by 0.55 m wide, with a nipple-type drinker in each crate. The animals had free access to the feeding area to consume water. During feeding times, the animals were confined in these crates (using a mobile gate) for 20 min without access to water. Communication between individual feeders (a drain tube) meant that if the animals used the drinker while eating, the water could have carried feed from one to another feeder. After 20 min of feeding, the drinkers were switched on again and all animals were released. The floor of the pen was solid concrete and covered by 12 rubber mats of 100 cm<sup>2</sup> and 30 mm in height (EBV 30 - Vedovati®). The pens were divided by 7 straight, non-electrified wires, with an access gate for each pen. The cleaning of the pens occurred daily in the morning. The mean temperature during the gestation period was 21.1 °C (maximum of 38.9 °C and minimum of 8.4 °C), the average humidity was 61.9%, and the rainfall was 534.6 mm (March 26th until October 31st). The average temperature during the lactation period was 22.7 °C (maximum of 38.9 °C and minimum of 8.5 °C), the average humidity was 61%, and the rainfall was 522.6 millimeters (August 1st to November 30th). The meteorological data were obtained at the Fernando Costa Campus station, approximately 1100 m from the pig farm.

#### 2.3. Experimental design

One animal was excluded for analyses because of an injury, prior the treatment assignment. Eighteen animals were fed a conventional diet, with low fiber (LFD) (2.53% crude fiber) and seventeen with high fiber diet (HFD) (12.86% CF), with a 35% inclusion of soybean hulls (Supplementary file A). The animals were divided into three blocks, according to the gestational period and each pen contained animals from both treatments, to avoid any social facilitation bias. Seven animals (1 HFD and 6 LFD) returned to estrus, and their data were excluded from the study. Therefore, for final data analysis, we evaluated the results of 12 gilts that received LFD treatment and 16 gilts that received the HFD treatment. To provide the same daily energy allowance, LFD animals received 2 kg of concentrate (3300 kcal per kg, calculated value) and HFD animals received 2.4 kg of concentrate (2764 kcal per kg, calculated value) per day, based on the National Research Council (2012). This daily allowance was divided into equal portions in two meals, at 8:00 a.m. and 3:00 p.m. The feed was prepared at the feed meal of the Fernando Costa Campus of the USP, in Pirassununga. The soybean hulls were ground to the same size as the other feed ingredients and mixed into the concentrate. The concentrate used was prepared at intervals of 15 days. In the farrowing pens, a standard lactation diet was used for both treatments and the sows had free access to the feed. The amount of feed consumed was not measured.

# 2.4. Data collection

Gilt weight was measured in the initial third (between 35 and 45 days) of gestation, in the middle third of gestation (72–82 days), at entry to the farrowing pen (107 days of gestation), at 21 days of lactation and at weaning, after 28 days of lactation. At the time of farrowing, the number of live, mummified, and stillborn piglets was recorded. Piglet weight was measured 24 h after birth, at 21 and 28 days of age. With this information, it was possible to obtain the total and average weight at birth, 21 days, 28 days and average daily weight gain of the litter and

individual average daily weight gain.

#### 2.5. Behavior

The measurements of the sows' behavior were obtained by direct observations performed on days 29, 30, 31, 59, 60, 61, 74, 75, 76, 89, 90, and 91, which correspond to the mean gestational age of each group. The observation protocol was adapted from the ethogram published by Zonderland et al. (2004), Tatemoto et al. (2019), and Martin and Bateson (2007). Table 1 includes the definition of behaviors present in the ethogram. Behavioral evaluations were performed by direct observation in the two feeding periods, during one hour before and one hour after feeding, totaling four sets of observations on each day of data collection.

Five trained observers participated in the data collection, taking turns between the pens during each period. All the observers were trained in a previous pilot study, in order to avoid any bias in data collection. The reliability among the observers was greater than 85%. The observation was performed by a combination of scan sampling, followed by a focal animal and continuous observation (Martin and Bateson, 2007), with each animal observed for 120 s in order to record the selected behaviors (see Table 1). Each animal was observed three times in each hour (giving six minutes at each observation pre and post feeding, morning and afternoon), totaling twenty-four minutes per day of observation. Each collection period included three consecutive days to avoid interference of possible events in the behavioral data (e.g. on days 29, 30, and 31). Moreover, we subsequently created two variables, by the combination of other observed behaviors. The behavior "rest" was created by the sum of lying ventrally and lying laterally. The behavior "oral" was created by the sum of sham-chewing, rooting the floor, licking the floor, interaction with fence or gate, and interacting with mats.

#### 2.6. Feeding motivation test

In the final third of gestation (at 100 days), a feed intake test was performed, based on the methodology described by Souza da Silva (2013). We prepared two bags with 2 kg of feed for each animal. Initially, 2 kg of food was provided (the first bag) for each animal and when almost all feed had been consumed, small extra portions of food were supplied gradually and individually (from the second bag), with a limit of another 2 kg of food. Therefore, the total amount for each test consisted between 2 kg (minimal) and 4 kg (maximum) of feed for each

Table 1
Definition of the behaviors of pregnant gilts fed a diet containing high fiber (12.86%) or low fiber (2.53%). The caption E indicates behaviors that were measured only as events and not as durations. This ethogram was adapted from Zonderland et al. (2004), Tatemoto et al. (2019) and Martin and Bateson (2007).

Behaviors	Definition
Sleep	Lying with the eyes closed
Lying ventrally	Lying with the belly facing the ground with the limbs under the body
Lying laterally	Lying sideways, with all the members extended laterally
Standing	Body supported by the four limbs
Sham-chewing	Continuous chewing without the presence of visible food in the oral cavity
Rooting the floor	Snout touches the ground followed by head movements
Licking the floor	Tongue touches the floor and is followed by movements with the head
Interacting fence or gate	Biting or nibbling the fence wire or gate
Interacting with mats	Snout or tongue touches mats followed by head movements
Bites (E)	Bite on any parts of the body (tail, vulva, ear, body) of a pen mate
Facing (E)	Face to face, with fixed view to the other animal
Pushing (E)	Pushing another animal using the head or the snout
Vocalization (E)	Sound emission emitted by the animal

animal. However, to avoid the effect of palatability or volume, all animals, irrespective of the treatment, received a mixed feed of each diet, conventional or high fiber. The test took place at the usual feeding site and lasted for 45 min. The final weight of residue in the second bag was measured to obtain the values of the total consumed. The animals had no access to water during the test.

#### 2.7. Saliva collection

The saliva collection was performed on the same days as the behavioral evaluation, that is, on days 29, 30, 31, 59, 60, 61, 74, 75, 76, 89, 90 and 91, corresponding to the average gestational age of each block. On all these days, two samples were collected per animal, the first between 6:00 and 06:30 a.m., the second between 6:00 p.m. and 6:30 p. m., to follow the circadian rhythm of cortisol. The methodology used was adapted from Siegford, Rucker and Zanella (2008) and Tatemoto et al. (2019), using hydrophilic cotton, two roller-shaped units (Apolo®), tied to a dental floss (DentalClean®) with long tips and presented to each animal. The animal chewed the cotton until it was saturated with saliva. The first sample collected was discarded; we repeat the protocol to collect only recently produced saliva. After the second sample was collected, it was introduced into a 15-ml falcon tube (Kasvi®), identified with the animal's number, time and day of collection. Subsequently, the tube was packed in an ice cube box until the end of the collection, destined for the laboratory and then frozen at  $-20~^{\circ}\text{C}$ until processing. The thawing was done in a container containing ice. After complete thawing of the sample, the sample was centrifuged for 2 min at 1000g (Celm Combate), and then the supernatant was aliquoted into 1.5 ml micro tubes (Kasvi®) and again frozen at -20 °C until analysis. This process assists in the removal of mucins and other components that may interfere with the analysis protocol. We used a cortisol enzyme immunoassay, without extraction, to measure the cortisol concentration (Palme and Möstl, 1997). All samples were analyzed in duplicates.

#### 2.8. Reproductive management and farrowing

The diagnosis of estrus was performed daily and those animals that returned to estrus more than twice, after the first artificial insemination, were not used in the study. Gilts were moved to the farrowing pens with, on average, 107 days of gestation. The farrowing was carried out in individual pens, measuring 4.3 m by 2 m, with sugar cane bagasse and hay for nest building and with lateral iron bars to protect the piglets against crushing. Each pen had a compartment made of masonry, measuring 0.97 m by 2.2 m, where the piglets had access to solid feed from the first day of birth. The creep area also had a bed composed of dehydrated sugarcane bagasse and the heating source was a 60 watt incandescent lamp. The farrowing pens were monitored using video cameras, with access via the internet, followed by direct observation after the onset of farrowing. Interventions were performed only when necessary, following a pre-established protocol, allowing a greater standardization of management.

## 2.9. Statistical analysis

The data were analyzed with the SAS software (Statistical Analysis System Institute, n.d.). Initially, the data were analyzed in relation to the presence of discrepant information (outliers) and normality of the residuals by the Shapiro-Wilk test. When the normality premise was not met, the logarithmic, square root or sine-arc transformation was used. The behavior interacting with mats was transformed by square root.

The behavioral data of duration and frequency were analyzed using the mixed model SAS procedure (PROC MIXED), in a factorial arrangement of treatments of type  $2 \times 4 \times 2$ , referring to two diets (high and low fiber), four gestational periods (30, 60, 75 and 90 days) and two different moments (before and after feeding). The analysis had the

collection days as nested parcels and the times of the day as sub-nested parcels.

For behavioral analyses, among the 15 different covariance structures tested, the one that best fit the statistical model was chosen based on the lowest value of the corrected Akaike information criterion (AICC) (Wang and Goonewardene, 2004). Data were submitted to analysis of variance and the model included the treatment effect, day and time, as well as the double and triple interactions as fixed factors and the block effect as a random factor.

Additionally to the mentioned statistical analyses, the gestational period effect data were decomposed into linear, quadratic and deviation effects of the quadratic effect using polynomial regression. The performance data of the gilts (all data included in Table 2) were submitted to the same preliminary evaluation regarding the normality of the residuals and presence of discrepant information, and later submitted to analysis of variance, having the effect of treatment as a fixed factor. The variable 'number of crushed piglets' was count data and was therefore analyzed using GLIMMIX procedure with a Poisson distribution. A significance level of 5% was adopted for all the tests performed.

#### 3. Results

#### 3.1. Behavior

There was an effect of feeding period (before and after feeding) on the duration and frequency of measured behaviors (P < 0.05). As expected, we found that the resting behaviors (sleep and lying laterally) were greater after feeding time, and activity behaviors (standing, sham chewing, rooting-floor, licking-floor, and interacting with mats) were greater prior to feeding time (Figs. 1 and 2). However, there was no treatment effect on any of the behaviors for duration or frequency. The animals performed the behavior 'sleep' and 'lying laterally' for longer (duration) and at higher frequency after feeding (Figs. 1 and 2).

For the frequency of sleep behavior there was interaction of treatment by time (P = 0.03). This interaction showed that the animals of both treatments slept more frequently after feeding, when compared to

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Variable	Treatment		Standard error mean	Probability
	High fiber	Low fiber	ciror incan	P value
Days of gestation	114.38	113.75	0.28	0.27
Total born	11.69	10.50	0.68	0.40
Born alive	11.12	9.33	0.65	0.18
Weaned	10.19	8.50	0.59	0.16
Crushed	0.87	1.17	0.25	0.45
Males born	5.12	4.50	0.40	0.45
Female born	5.06	4.00	0.32	0.10
Gilt weight in the initial third of gestation (kg)	174.85	168.10	2.55	0.19
Gilt weight in the middle third of gestation (kg)	203.00	193.45	2.13	0.02*
Gilt weight at 109 days of gestation (kg)	216.25	208.73	1.86	0.04*
Gilt weight at 21 days of lactation (kg)	199.38	201.44	2.55	0.71
Gilt weight at 28 days of lactation (kg)	196.87	193.00	3.32	0.58
Total litter weight at birth (kg)	18.02	16.09	0.76	0.21
Mean weight at 24 h after birth (kg)	1.68	1.82	0.48	0.14
Total litter weight at 21 lactation (kg)	62.31	57.28	3.10	0.43
Total litter weight at 28 days of lactation (kg)	84.97	84.60	3.02	0.95

the moment before feeding, but the difference was greater for LFD animals.

There was an interaction of treatment by time on duration (P=0.0041) and frequency (P=0.0128) of sham-chewing behavior. This interaction showed that LFD animals sham chewed longer and more frequently before feeding (Fig. 3).

For the duration of licking behavior, there was a triple interaction between treatments, day and time. After more detailed analysis of the interaction, it was identified an interaction of treatment and meal period was shown only at day 30 of gestation. Thus, we identified that prior to feeding on this day, the LFD treatment sows licked the floor longer than the HFD treatment sows (Figs. 4 and 5). The data for the triple interaction for the frequency of behavior 'licking ground', behaved similarly to duration.

# 3.2. Feeding motivation test

We did not find treatment effects on the total of consumed feed during the feeding motivation test (p = 0.34). Data on the ad libitum feed intake test show that there was no treatment effect for feed consumed during the test (LFD =  $2926.67 \, \text{g}$ , SEM 153.89; HFD =  $3125.26 \, \text{g}$ , SEM 133.27).

#### 3.3. Performance

For the performance data (see Table 2), the only difference we found was that HFD gilts were heavier in the middle third and at 109 days of gestation (day of moving to farrowing pens).

#### 3.4. Salivary cortisol concentration

There was no effect of treatment or day on salivary cortisol concentration (Supplementary File B).

For the salivary cortisol data, the only effect observed was regarding time of day, where the samples from afternoon showed higher concentration (p = <0.0001; morning samples = 212.98 pg/50  $\mu$ l; afternoon samples 35.32 pg/50  $\mu$ l). We did not identify a treatment effect on the diurnal pattern.

## 4. Discussion

The use of fiber to mitigate the impact of hunger in pregnant sows has been investigated for many years. Nonetheless, most of those studies were carried out with crate-housed sows, a well know source of stress for the animals. We studied animals in a group housed scenario and we used a high-quality fiber (NRC, 2012) as a strategy to mitigate prenatal stress and to understand the consequences to the gilts, and to their offspring (Bernardino et al., 2016).

In this study, we showed that a high fiber diet during gestation reduced stereotypic behavior but did not affect performance of gilts. We found differences in many of the measured behaviors, comparing pre and post feeding time. The animals showed much more resting behavior after feeding time, regardless of the treatment. Moreover, we found an interaction between treatments and time, where the animals from both treatments slept more after feeding time compared with prior to feeding time, but with a more marked difference for LFD gilts. Other research, Zonderland et al. (2004) and Souza da Silva et al. (2012), identified similar findings, as their animals showed more rest and inactive e behaviors after feeding time. In one study (Zonderland et al., 2004), an effect of the treatment on lateral-lying behavior was identified, indicating that the animals that received high fiber diet spent more time in this position, which may be indicative of better satiety, an effect not identified in this study. However, one important issue that needs to be addressed is the fact that we did not measure the amount of feed consumption during the lactation period. This is a limitation of this study and this information would benefit our data set.

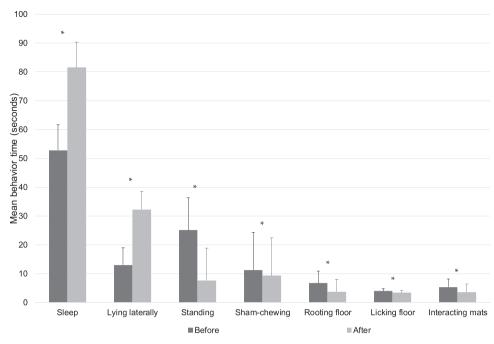


Fig. 1. Duration of behaviors that presented difference in relation to the moment of observation (before and after feeding time) in group-housed pregnant gilts fed with diet containing high or low fiber. Aggregate data from the 28 gilts from both treatments. \* Indicates difference between feed moments in both treatments (P < 0.05).

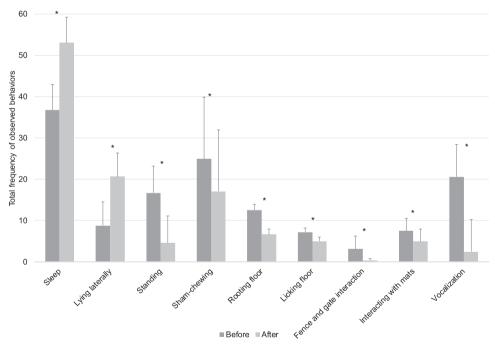


Fig. 2. Frequency of behaviors that differed in relation to the moment of observation (before and after feeding time) in group-housed pregnant gilts fed with diet containing high or low fiber. Aggregate data from the 28 gilts from both treatments. \* Indicates difference between feed moments in both treatments (P < 0.05).

One important result of our study was regarding the stereotypic behavior: sham chewing. Sows from the LFD treatment sham chewed longer and more frequently before feeding. Souza da Silva et al. (2013) found similar results to ours. In their study, the sows of the HFD treatments presented lower stereotypic chewing behavior at the time prior to evening feeding (treatment and time interaction). Sham chewing behavior is defined by the action of continuous chewing without food content in the month cavity (Zonderland et al., 2004). This behavior of chewing in a stereotypic way (continuous, with no apparent function, and repeatedly performed) can be explained by different reasons. It

could be a compensatory effect, anticipating the lack of satisfaction of chewing needs due to the small volume of the next feeding (De Leeuw et al., 2008), or due to hunger (Appleby and Lawrence, 1987), long-term confinement and age (Zhang et al., 2017). However, during the feed motivation test, we were not able to identify differences in sows' feed motivation based on their dietary fiber treatment. Regarding the bulk effect, we fed HFD sows with 20% more feed than the control group. This amount, despite providing higher fiber and bulk, probably was not enough to meet their chewing need, since their ad libitum intake is higher (D'Eath et al., 2009). Information about initial feeding

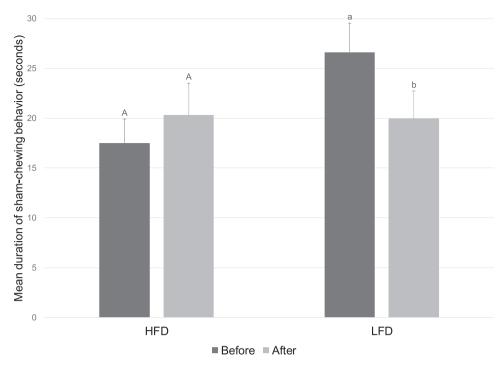


Fig. 3. Effect of interaction between treatment and time for duration of sham-chewing behavior in group-housed pregnant gilts, separated by treatment, fed with diet containing high or low fiber, before and after feeding. High fiber, N = 16; low fiber, N = 12. Letters that differ in columns = P < 0.05.

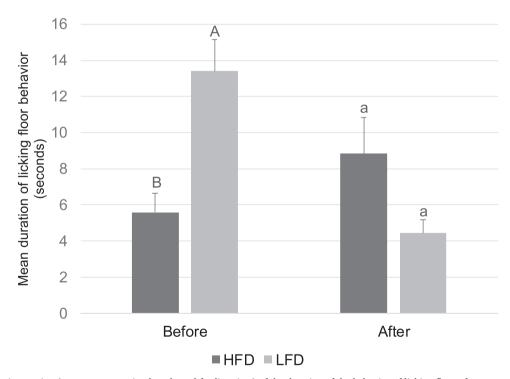


Fig. 4. Effect of triple interaction (treatment, gestation length, and feeding time) of the duration of the behavior of licking floor of pregnant gilts group housed fed with diet containing high or low fiber. Interaction of treatment and hour only at day 30 of gestation (the presented data in this figure). Different letters indicate difference. Aggregate data from the 28 gilts. Value of P = 0.0141.

motivation could also be an alternative method to allocate the animals between the treatments, in order to minimize the variation among groups. In the reported results, there was only an effect of the interaction between treatment and time for sham chewing behavior and not for other behaviors. In previous studies, pigs fed diets with low fiber contents showed more stereotypic behavior after feeding (Bolhuis et al., 2010; De Leeuw et al., 2008; Jiang et al., 2019; Meunier-Salaün et al.,

2001; Souza da Silva et al., 2012), or the manifestation of stereotypic behaviors did not differ in sows fed with fiber-rich diets or not (Holt et al., 2006). It is important to highlight that this research is one of the few studies that kept sows in a group-housing system to study the effect of fiber consumption on animal welfare indicators. In our previous study we showed that stereotypic behavior did not affect productivity (Tatemoto et al., 2019), supporting the outcome that this welfare indicator

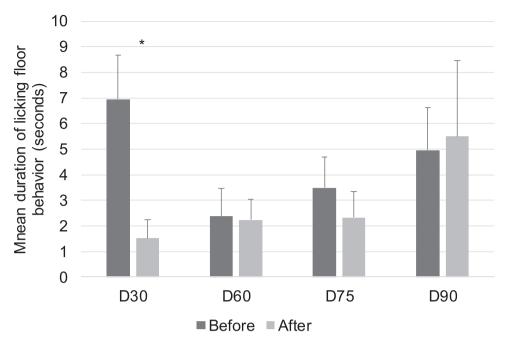


Fig. 5. Effect of the triple interaction (time, treatment, and days of gestation) of the duration of the licking floor behavior of pregnant gilts group housed fed with diet containing low fiber. We found the triple interaction only at the 30th day of gestation. Interaction between day and time for LFD sows. \* P = 0.0012.

was not related with performance.

We consider that the behavioral differences identified, such as in sham chewing, are valid as indicators of poor welfare (Mason, 1991; Tatemoto et al., 2019). A possible explanation for the limited effect of treatment on the duration and frequency of the other behaviors studied was that gilts of the same treatment were housed in the same pen. This may have influenced posture of the activities of the animals, through social facilitation or direct effects on the animals' activities. However, this was our only option to minimize a confounding effect of housing on the observed variables, given the limited number of animals studied. Furthermore, the quality of the fiber from soybean hulls is high and may be less bulky than fiber from other sources, like hay or silage. High quality fiber in the diet has only a small effect on welfare, in comparison with the large effect if the fibrous material can be manipulated. Nevertheless, this approach was not possible because we needed to control the exact amount of fibrous material that was ingested. In addition, it is more beneficial for farmers, and sustainable, to use a local feed source, particularly if it is a by-product from local or national industry.

Our performance data are not so revealing. We found that the sows from HFD treatment were heavier at 109 days of gestation. Some studies have shown opposite results, studying different sources of fiber, such as 20% of soybean hulls (Darroch et al., 2008), 35% of soybean hulls (Gentilini et al., 2004), and other sources of fiber (Holt et al., 2006). Renteria-Flores et al. (2008) found similar results to ours and attributed them to the greater energy utilization of the high fiber diet, even if isoenergetic.

The few differences that we found in the productivity data are similar with previous publications (Budiño et al., 2014; Danielsen and Vestergaard, 2001; Gentilini et al., 2004; Holt et al., 2006; Mcglone and Fullwood, 2001; Renteria-Flores et al., 2008). Contrary to this study and the studies mentioned above, Veum and collaborators (2009) found that wheat fiber addition to the diet of pregnant sows led to production of more piglets and heavier piglets at weaning. They also identified higher feed intake in lactation period, a fact that is related to the longevity of the sow. Jiang et al. (2019) found greater litter size and piglets born alive from sows fed with 7.5% of crude fiber. Additionally, the same study evaluated the fecal microbiome; animals that received more fiber showed an increased diversity and richness of the fecal microbiome.

These differences in the outcomes of the studies could be due to differences in the kind of fiber. In our study, we analyzed the litters from 28 gilts and a higher number of animals might have revealed different outcomes. In other words, it is possible that the power of this experiment was not great enough for clarifying statistical differences between the treatments.

We did not find any differences between the treatments in salivary cortisol concentration. A review by D'Eath et al. (2009) states that it is difficult to interpret the results of greater activity of the pituitary-adrenal axis in cases of feed restriction or hunger, since some studies showed higher, equal or lower values of glucocorticoids. Moreover, the plasma cortisol concentration in gilts kept in gestation crates or kept outdoors, under high or low level of dietary fiber treatments, did not differ (Mcglone and Fullwood, 2001). No difference in salivary cortisol concentration was identified, because salivary cortisol is not a good measure of long-term welfare (Broom, 2017; Broom and Fraser, 2015). In addition, there may be a difference depending on the sampled matrix for cortisol analyses as Jiang et al. (2019) showed a different result for salivary cortisol and fecal cortisol metabolites. Only an effect of the time of sampling (morning vs afternoon) was identified. In diurnal animals, the salivary cortisol concentration is higher in the morning. Following the circadian rhythm, with a morning and afternoon sampling, can give us information on HPA homeostasis in sows (Zanella et al., 1998).

We did not find any treatment effect on the feeding motivation test. Results are in contrast to a previous study published by Souza da Silva et al. (2013), who performed an unlimited feed intake test and found lower feed motivation in sows fed with high fiber diets. Robert et al. (1997) also found smaller values for feed motivation in a test in pregnant sows fed high fiber diets.

Another study (Ramonet et al., 2000) also used the practice of operant conditioning and found there was no difference for rewards obtained by sows fed with fiber-rich diets (one containing beet pulp and the other with wheat bran) or cornstarch. Jensen et al. (2015) also found no difference in pregnant sows fed diets with 35% or 18% dietary fiber in the operant conditioning test, although they found physiological and behavioral differences indicative of reduced hunger in animals fed fiber-rich diets. It is important to mention that the amount of fiber used in previous experiments was considerably higher than that reported in

this experiment (Bergeron et al., 2000: 23% dietary fiber; Jensen et al. (2015): 35% dietary fiber). The reason for our choice was due to the low metabolizable energy of soybean hulls. If we used a larger amount, it would be necessary to add oil, which would alter the palatability of the feed and would compromise the replicability of the study. Differences between the reported work and previous research may be related to the fact that some studies used gilts (Robert et al., 1997; Souza da Silva et al., 2013) and others multiparous sows (Bergeron et al., 2000; Jensen et al., 2015; Ramonet et al., 2000), since there is a large difference in appetite between these animals (Jensen et al., 2015). In our study, only gilts were assessed, avoiding the bias of age and previous experience of farrowing.

Possibly, a previous standardization of the groups in relation to the animals' feed motivation would be more informative in relation to effects of dietary treatment on satiety. The individual profile of the sows in relation to their feed motivation may have been a factor that contributed to the absence of differences in the results. A feeding motivation test, prior to the beginning of the experiment, would enable this standardization and facilitate a greater homogeneity of the replicates.

## 5. Conclusions

The increased expression of stereotypic behavior by gilts fed with a low fiber diet may be an indicator of a compensatory mechanism to meet the needs of chewing and feed intake. These behaviors are often related to poor welfare, perhaps associated with hunger in those animals. However, the animals fed a high fiber diet gained more weight during gestation, which may be indicative of greater utilization of dietary energy, although the diet rich in soybean hulls did not alter the productive performance of the gilts.

## Acknowledgements

We are grateful to the Department of Preventive Veterinary Medicine and Animal Health and to the School of Veterinary Medicine and Animal Science. We would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq), for the scholarships that were provided. We are also grateful to Beate von Staa, from Araporanga Farm, Jaguariaíva, for the collaborative work.

# Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2021.105433.

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