

USING LIGNOCELLULOSE FIBER IN FEED TO MAINTAIN HEALTH AND IMPROVE THE GROWTH OF POST-WEAN PIGLETS

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Supporting Information

ABSTRACT: This study aims to determine how adding fiber lignocellulose in feed affects performance, intestinal health, morbidity, and mortality in post-wean piglets. The animal model used was 54 piglets divided into three groups of treatments: the control group was given starter commercial feed, group T1 was given starter pig feed with added 1% lignocellulose fiber, and the T2 group was assigned starter feed with the additional 2% lignocellulose fiber. Treatment is given for 4 weeks starting at 41-68 days old. Health observations were also carried out during the research. Piglet performance is determined based on feed intake, body weight and feed conversion ratio (FCR). Examination of the health of the gastrointestinal tract by measuring the length and weight of the intestine, microscopic length of the villi and depth of the crypts, as well as the number of *Escherichia coli* in the piglet's small intestine. The research showed that adding 1% and 2% lignocellulose fiber may significantly lower diarrhea incidents, and death was not found in the treatment group. Lignocellulose fiber may increase villi's intestinal length and weight height compared with control. It can be concluded that supplementing lignocellulose fiber through feed may increase the piglet's health growth and efficiency.



Keywords: Body weight, Gut health, Lignocellulose fiber, Performance, Post-wean piglet.

INTRODUCTION

Early weaning of piglets is often accompanied by stunted growth and severe diarrhea (Campbell et al., 2013). In the first three days, as many as 25% of post-wean piglets reported experiencing anorexia, stress and nutrition deprivation so that the condition becomes weak and vulnerable to various disease infectious (Pensaert and Martelli, 2016; Eriksen et al., 2021). It is known that the main etiology of this event is multi-factorial, such as anorexia and malnutrition in piglets' gastrointestinal disorders include changes in the small intestine's structure and enzyme activity (Pickard, 2003; Kobek-Kjeldager, et al., 2022). Recent data show a transient increase in intestinal wall mucosal permeability, disturbance of the electrolyte-secretory-absorptive balance and local changes in inflammatory cytokine patterns after weaning (De Groot et al., 2021; Tang et al., 2022; Michiels et al., 2023). Here, new insights into the interactions between feed components, commensal microbiota and the physiology and immunology of the host gastrointestinal tract need to be gained, and several new dietary strategies have been studied that focus on improving gut health (Danneskiold-Samsøe et al., 2019). Prebiotics and probiotics are obvious nutritional choices, as are other bioactive substances of plant origin (Lalle's et al. 2007). Using carboxymethylcellulose fiber mixed into piglet feed after weaning can protect the gastrointestinal tract from colibacillosis infection (McDonald et al. 2001; Lalle's et al. 2006). Adding this fiber is thought to stimulate the growth of gastrointestinal villi in piglets (Van Nevel et al., 2003). The use of fiber in feed for piglets after weaning is vital to maintain digestive tract health, intestinal motility, and animal welfare (Bosse, 2020). Fiber content in feed must be included to maintain the physiological function of the livestock's intestines. Nutritionists have long been aware of the importance of fiber in pig feed, but there are not many positive studies on the use of fiber. One of the fibers that is easy to obtain is lignocellulose, whose main components are cellulose, hemicellulose, and lignin. For this reason, this research aims to determine the effect of adding lignocellulosic fiber in post-weaning piglets' feed on feed consumption, body weight, feed efficiency and gastrointestinal health by looking at the length and weight of the intestine, the height of the villi and the depth of the crypts of the jejunum and ilium as well as the presence of gastrointestinal E. coli. It is also hoped that we can find out the optimal dose for using lignocellulose fiber for weaning piglets (Bosse, 2020).

This research aimed to determine how adding fiber lignocellulose in feed affects performance, intestinal health, morbidity, and mortality in post-wean piglets.

MATERIALS AND METHODS

Experiment design

A total of 54 post-weaning landrace piglets, in healthy condition, with an average weight of 15 kg, were divided into three groups, namely T0 as a control which was given starter pig feed. Treatment group T1 was given starter feed plus 1% lignocellulose fiber, and T2 was given starter feed with supplementation of 2% lignocellulose fiber. Treatment was given for 28 days, starting from piglets aged 41-64 days. The lignocellulose fiber provided combines Cellulose, Hemicellulose and Lignin in balanced proportions. The nutritional content of the starter pig feed used contains a maximum water content 13%, protein 21-23%, fat minimal 5%, fiber maximal 5%, ash content maximal 7%, calcium minimal 0.9%, phosphorus min 0.6%, and Metabolizable Energy (ME) 2900-3000 Kcal/kg.

After the treatment period ended, all piglets were given 551 starter feed until 68 days of age (the end of the starter phase). The amount of drinking water given is as needed. The piglets' initial body weight data was measured the day before they were given treatment, while the piglets' body weight data after treatment was measured at the end of the study, namely 68 days of age. Weight gain is calculated as final body weight minus initial body weight. The amount of feed used is done by weighing the feed given daily and the remaining feed given. The amount consumed is the amount given minus the remaining feed per day. The total feed spent during the study was obtained by adding daily feed. The Feed Conversion Ratio (FCR) calculation divided the total feed consumed during the study by body weight gain.

Morbidity data was observed for clinical symptoms such as diarrhea and mortality from the time the treatment was given until the end of the study. Data Isolation and identification of bacteria was carried out by taking fecal samples with a rectal swab, which were then placed in transport media to be taken to the laboratory after one week of treatment. Data on the weight and length of the intestine and the length of the small intestine's villi were obtained at the end of the study. Pigs that have received treatment are slaughtered, their intestines are taken, the feces are removed, and the length (in cm) and the weight of the intestine (in grams) are measured. Histological preparations were made and examined under a microscope to measure the length of the villi and crypts of the jejunum and ilium (Yin, 2001). Data on the amount of feed consumption, body weight gain and FCR were analyzed using the Variety Test. If there was a real effect, the analysis was continued with the Duncan Multiple Range Test. Meanwhile, morbidity, mortality, small intestine wall thickness, villi length and E. coli isolation were analyzed descriptively.

RESULTS AND DISCUSSION

Pig performance

Pig performance was measured with count amount of feed consumption, increase of body weight and Feeds Conversion Ratio (FCR). The performance of piglets during the study can be seen in Table 1. According to Table 1, the control group (lignocellulose 0%) consumed significantly less feed, totaling 47.56 kg, from 41 to 68 days of age compared to the groups given lignocellulose 1% (49.25 kg) and lignocellulose 2% (50.96 kg). However, there was no significant difference in feed consumption between the lignocellulose 1% and 2% groups. Lignocellulose, a type of crude fiber that does not dissolve in water, can increase the amount of feed consumed by piglets. This fiber affects intestinal function by expanding and speeding up intestinal peristalsis, which reduces constipation and endotoxin impact on gut health. Adding vegetable fiber to piglet feed can also promote gastrointestinal development in terms of size and functionality (Van Hees et al., 2019; Vastolo et al., 2022).

Table 1 - Influence of lignocellulose Fiber Addition in feed for piglets aged 41-64 days to performance.				
Treatment	Feed intake (Kg)	Body weight gain (Kg)	FCR	
Lignocellulose 0% (N=18)	47.57ª	15.93ª	3.00 ^a	
Lignocellulose 1% (N=18)	49.25 ^{ab}	18.22 ^b	2.75 ^b	
Lignocellulose 2% (N=18)	50.96 ^{bc}	20.10°	2.56°	
Same letters on the same column: no significant difference (p > 0.05). The lignocellulose used is Arbocel Rc-fine Arbocell contains 65% fiber consisting of hemicellulose, cellulose and lignin. Last weighing done at 68 days old.				

According to this research, an increase in feed intake leads to increased body weight in piglets. The study found that piglets fed with 1% lignocellulose gained an average weight of 18.22 Kg, while those fed with 2% lignocellulose gained 20.10 Kg between 41–64 days. These values were significantly higher than the control group which gained 15.93 Kg. Additionally, the group given 2% lignocellulose showed a greater increase in weight than the group given 1%. Insoluble fiber in feed such as barley husk has been found to enhance intestinal morphology, improve villi length, and activate

enzymatic mucosa, unlike soluble fiber (such as pectin) found in feed. The number of effects of insoluble fiber lignocellulose (Arbocel RC-Fine) are increased growth hormone (GH), Insulin-Like Growth Factor-1 (IGF-1), Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3), which can decrease pro-inflammatory cytokines, IL-6 gene expression but not TNF- α and IL-1 β (Superchi et al., 2017).

This study demonstrated that the group of piglets fed with 2% lignocellulose had a significantly lower FCR of 2.56 compared to the group fed with 1% lignocellulose with an FCR of 2.75. Additionally, the group fed with 1% lignocellulose had a significantly lower FCR than the control group with an FCR of 3.0. These findings suggest that piglets that consume more food tend to produce more meat.

Morbidity and mortality

Results of piglet observation after giving lignocellulose in feed to incident mortality and morbidity can observe in Table 2. According to Table 2, the control group had a morbidity rate of 83.3%, while the group given 1% lignocellulose had a morbidity rate of 55.6%. The group given 2% lignocellulose had a morbidity rate of 50%, with diarrhea being a common symptom. These findings suggest that adding lignocellulose to animal feed may not be effective in preventing diarrhea, which is in agreement with Silva-Guillen et al. (2022). The exact cause of diarrhea, whether it is due to a bacterial infection or other factors, remains unknown, and further research is needed. In this study, all subjects tested positive for bacteria in their feces, such as *E. coli*, Staphylococcus sp, and Bacillus sp. However, additional studies are required to determine if these bacteria are responsible for causing sickness.

Table 2 - Influence of lignocellulose in feed for piglets aged 41-68 days to morbidity and mortality.

Dosage	Morbidity (n, %)	Mortality (%)	Remarks
0% Lignocellulose (n=18)	15 (83.3)	0	diarrhea
1% Lignocellulose (n=18)	10 (55.6)	0	diarrhea
2% Lignocellulose (n=18)	9 (50.0)	0	diarrhea

Isolation and Identification of Bacteria from the intestines of pigs after being given lignocellulose

Bacteria in piglet intestines were isolated and identified before and after lignocellulose treatment using fecal samples (Table 3). Table 3 displays the presence of bacteria like *E. coli, Bacillus sp,* and *Staphylococcus sp* in all subjects' feces. Lignocellulose in feed may be ineffective in killing the bacteria in the digestive tract.

Table 5 - Types Bacteria present in feces before and after being given ingnocendiose in feed.						
No	0% Lignocellulose		1% Lignocellulose		2% Lignocellulose	
1	Before	After	Before	After	Before	After
2	Bacillus sp	Bacillus Sp	E.Coli	E.coli	E.coli	E.coli
3	E.coli	E.coli	E.coli	Ecoli	E.coli	E.coli
4	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
5	E.coli	E.coli	E.coli	E.coli	Staphylococcus sp	Staphylococcus sp
6	Ecoli	E.coli	E.coli	E.coli	E.coli	E.coli
7	E.coli	E.coli	Staphylococcus sp	Staphylococcus sp	E.coli	E.coli
8	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
9	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
10	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
11	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
12	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
13	Ecoli	E.coli	E.coli	E.coli	E.coli	E.coli
14	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
15	Ecoli	E.coli	E.coli	E.coli	E.coli	E.coli
16	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
17	E.coli	E.coli	E.coli	E.coli	E.coli	E.coli
18	E.coli	E.coli	E.coli	E.coli	Bacillus sp	Bacillus sp

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Length and weight of subjects' small intestines

According to Table 4, subjects who were given 1% or 2% lignocellulose from ages 41-64 days had significantly heavier intestinal weights (0.9 kg and 1.2 kg, respectively) than the control group (0.6 kg) (P<0.05). However, there was no significant difference in intestinal weight between pigs given 1% and those given 2% lignocellulose (P>0.05). When the dose of lignocellulose was increased to 2% of the actual length of the intestine, there was a significant increase in length (17.30 meters) compared to the control (P<0.05). However, there was no significant difference in length compared to pigs given 1% lignocellulose. Therefore, it seems that increasing the dose of lignocellulose to 2% did not significantly increase intestinal weight compared to the 1% dose. Given lignocellulose, intestinal length and weight increase, resulting in increased absorption of feed, which has a positive impact on body weight increase.

Table 4 - Influence of lignocellulose in feed addition on the length and weight of pig intestines.				
Treatment	Small Intestine length (m)	Small Intestine weight (kg)		
Lignocellulose 0%	13.42 ª	0.6ª		
Lignocellulose 1%	15.36 ^{ab}	0.9 ^{ab}		
Lignocellulose 2%	17.30 ^b	1.2 ^b		
* the same letters in the same column showed no significant difference (p > 0.05)				

Histology of villi height, jejunum crypt and llium from subject intestine

Based on the histological analysis of the jejunum and ileum of pigs aged 68 days, there was an increase in the height of villi when they were fed with lignocellulose from 41 to 64 days of age. However, the height of the crypts in both areas appeared to be irregular. This information is available in Table 5.

The histology of the villi in the jejunum and ileum of pig intestines were examined in Table 5. Pigs that were not fed lignocellulose had shorter villi (as seen in Figures 1 and 2), but when given 1% lignocellulose, their villi and ilium increased in height (Figures 3, 4 and Figure 5). Increasing the dose of lignocellulose to 2% further increased the height of the villi and ilium (Figures 6 and 7). This indicates that the small intestine's area for nutrient absorption is wider, allowing for optimal absorption of nutrients. The jejunum is located between the duodenum and ileum, and it is responsible for absorbing almost 90% of the nutrition from digested food (Wijtten et al., 2011; Roura et al., 2022). It represents two-fifths of the whole small intestine's length, while the duodenum is responsible for digestion chemistry (Roura et al., 2022).

Nutrient absorption primarily occurs in the small intestine, specifically in the jejunum. The epithelial cells, villi, and microvilli that make up the surface of the small intestine enhance its absorption capabilities (Mosenthin, 1998). The jejunum is specifically responsible for the absorption of nutrients, such as peptides, amino acids, vitamins, and glucose, which are transported throughout the body through active and passive transportation. Temporary fructose is transported passively. The ileum, the third part of the small intestine, extends from the jejunum to the ileocecal valve and is almost three-fifths the length of the entire small intestine. By adding lignocellulose to pig feed, the length of the villi and ileum can increase, creating a wider absorption area for nutrients and promoting pig growth, which is in agreement with findings of Silva-Guillen et al. (2022) in pigs and Bogusławska-Tryk et al. (2020) in chickens.

Lignocellulose level (%)	Villi height (µm)		Crypt height (µm)	
-	Jejunum	lleum	Jejunum	lleum
Lignocellulose 0%	323.70ª	326.84ª	435.18ª	404.07ª
Lignocellulose 1%	418.60 ^b	485.02 ^b	295.50 ^{ab}	374.89 ^{ab}
Lignocellulose 2%	478.85°	508.98°	435.18°	384.46 ^{bc}
* The same letters in the same column showed no significant difference (p > 0.05)				

Table 5 - Influence of lignocellulose in feed addition height pig intestine villi and crypts (100x magnification).



Figure 1 - Overview of Jejunum Villi length without lignocellulose in feed/control group (100x)



Figure 2 - Overview of Ileum Villi length without lignocellulose in feed/control group (100x)



Figure 3 - Overview of Jejunum Villi length with 1% Lignocellulose in feed (100x)



Figure 4 - Overview of Ileum Villi length with 1% lignocellulose in feed (100x)





Figure 6 - Overview of Ileum Villi length with 2% lignocellulose in feed (100x)

CONCLUSION

According to the findings, adding 1% or 2% lignocellulose to feed for pigs aged between 41 and 64 days can result in a significant increase in body weight and feed consumption and a drastic reduction in FCR. Lignocellulose can also help prevent mortality in pigs, however, it cannot prevent morbidity or diarrhea incidents completely, nor can it kill all the bacteria in their intestines. Nevertheless, diarrhea and bacteria in the intestines do not affect the small intestine's anatomy and histology. In fact, adding lignocellulose to their diet can significantly increase the weight and length of the intestine and the length of villi and crypts in the small intestine.

DECLERATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical consideration

This research has been approved by the Animal Commission Ethics Faculty Udayana University Veterinary Medicine with certificate agreement Number B/40/UN14.2.9/PT.01.04/2023.

Author contribution

All authors contributed equally to the study.

Competing interests

The authors declare no competing interests in this research and publication.

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