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<b>Title (English)</b>	Investigation of supplementation with a combination of fermented bean dregs and wheat bran for improving the growth performance of the sow
<b>Running Title (English)</b>	fermented mixture of bean dregs on sow's performance
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6 **Investigation of supplementation with a combination of fermented bean dregs and wheat bran for**

7 **improving the growth performance of the sow**

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15 **ABSTRACT**

16 To investigate the effect of dietary supplementation with a fermented mixture of bean dregs and  
17 wheat bran (FBW) on sow performance. FBW was given to sows during late gestation and lactation; in  
18 total, 24 sows were randomly assigned to 4 groups (control diet; 3% FBW diet; 6% FBW diet; 9% FBW  
19 diet, n=6). The weight ratio of bean dregs (wet) to wheat bran was 4:6. Sows were fed different diets  
20 from 85 d of gestation until weaning. The results showed that supplementation with FBW increased  
21 average daily feed intake (ADFI) during lactation ( $p < 0.05$ ). FBW supplementation also increased litter  
22 weight and milk yield ( $p < 0.05$ ). The contents of *Escherichia coli* in the feces of the treatment groups  
23 were significantly reduced by FBW supplementation ( $p < 0.01$ ). FBW supplementation significantly  
24 improved the fecal morphology ( $p < 0.05$ ), alleviating sows' constipation. In conclusion, FBW could  
25 increase the ADFI, improve lactation and piglet litter weight in sows and reduce the pathogenic bacterial  
26 content in sow feces and constipation.

27 **Keywords:** Fermented mixture of bean dregs and wheat bran, Metabolite, Sow, Piglet growth

28

29 **INTRODUCTION**

30 Soybean meal is the major protein source in the pig diet. In recent years, because of continuous  
31 increases in soybean meal prices and transgenic crop safety problems, alternative protein sources,  
32 including bean dregs, have been widely sought to reduce the content of soybean meal in feed [1, 2]. In  
33 the process of making soy milk and tofu, soybeans are first mechanically crushed and then cooked, and  
34 the residue left over from the final filtration is soybean dregs [3]. The dry matter of bean dregs contains  
35 27% protein and 53% carbohydrate, which contains more fiber and less protein than soybean meal [4, 5].  
36 Bean dregs contain easily decomposable carbohydrates and amino acids and are rich in carbon and  
37 nitrogen [6]. China produces over 80,000 tons of bean dregs annually, and most bean dregs are discarded  
38 [7]. However, trypsin inhibitors, the major anti-nutritional factor in soybean dregs, can reduce the  
39 digestion and absorption of dietary protein [8]. Reducing the anti-nutritional factors in soybean dregs has  
40 become a significant problem in the utilization of soybean dregs.

41 Feed fermentation is a promising solution to reduce these adverse effects of trypsin inhibitors [6, 9].  
42 Because bean dregs contain insoluble fiber and 70% moisture, wheat bran was added to reduce the  
43 moisture to 45%, which was suitable as a substrate for solid fermentation [10]. Wheat bran also contains  
44 a moderate quantity of starch that can contribute to the fermentation of the mixed product. Fermentation  
45 by *Bacillus subtilis* or *Aspergillus oryzae* can significantly enhance the relative content of crude protein  
46 [11, 12]. It can degrade antigenic proteins and trypsin inhibitors in soybean protein [13]. Allergic  
47 reactions and immunoreactivity induced by soybean protein can be reduced by microbial fermentation  
48 [14]. Furthermore, oligosaccharides extracted from fermented bean dregs significantly reduced the  
49 concentration of ammonia nitrogen and pH and elevated short-chain fatty acid levels in imitation gut  
50 fermentation [15], which showed that fermented bean dregs could have a prebiotic function.

51 The profitability of large-scale pig farms depends on sow productivity, including litter size, piglet

52 weight, and sow reproductive performance [16]. Nutrition in the maternal diet plays a vital role in fetal  
53 development and offspring growth, making it an essential factor to consider when feeding sows. The  
54 fetus gains weight rapidly in late gestation (GD), which is the critical period for fetal growth [17, 18].  
55 We chose to intervene nutritionally in sows during late GD.

56 This study aimed to assess the effect of a fermented mixture of bean dregs and wheat bran (FBW)  
57 in compound feed for sows during late GD on production parameters, nutrient digestibility, colostrum  
58 composition, fecal microbial flora, and constipation.

## 59 MATERIALS AND METHODS

60 This experiment was approved and conducted under the supervision of the Animal Care and Use  
61 Committee of Nanjing Agricultural University (Nanjing, Jiangsu Province, China). All animals were  
62 raised and maintained per the Animal Care and Use Guidelines of Nanjing Agricultural University  
63 (SYXK (Su) 2011-0036).

### 64 Animal, diets, and housing

65 The experiment was conducted on a pig breeding farm (Suqian Municipality, Jiangsu). A total of 24  
66 sows (PIC, Camborough) at GD 85 with parities of  $5.98 \pm 0.41$  were selected based on body weight (BW)  
67 ( $228.75 \pm 5.4$  kg), and they were assigned to 4 groups (n=6). The dietary treatments were 1) Control  
68 (CON; diet without FBW), 2) FBW3 (CON+ 3%FBW), 3) FBW 6 (CON+ 6%FBW), and 4) FBW 9  
69 (CON+ 9%FBW). The FBW used in this trial was fermented by bean dregs and wheat bran with *B.*  
70 *subtilis*, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *Enterococcus faecalis*. The weight  
71 ratio of bean dregs (wet) to wheat bran was 4:6. The 4:6 ratio was DM-based. After the raw materials  
72 were fully mixed, the mixed bacterial fluid was inoculated, and the inoculation proportion was 6%. The  
73 proportions of *L. acidophilus*, *S. cerevisiae*, *B. subtilis*, and *E. faecalis* in the mixed broth were 2:2:1:1.

74 The initial water content of the fermentation was 45%, the fermentation tank was sealed. First, ferment  
75 at 35 °C for 4 hours, then the fermenter heats up to 37 °C and continues to ferment for 8 hours, then the  
76 fermenter cools down to 30 °C and continues to ferment for 36 hours. The chemical analysis of FBW is  
77 presented in Table 1. The basal diet was formulated according to NRC (2012) for gestating and lactating  
78 sows. FBW was supplemented when it was wet but calculated as an air-dry condition. The ingredients  
79 and compositions of the diets are provided in Table 2.

80 The sows were fed different diets from GD 85 to 19 of lactation (LD) when the piglets were weaned.  
81 The average day for the gestating period was 114 days. The sows were provided with 3 kg of feed every  
82 day from GD 85 to GD 111, and they were fed twice daily at 06:30 and 15:30. Then, the feed allowance  
83 was reduced by 0.5 kg/d until the farrowing day. Sows were fasted on the farrowing day and were fed 2  
84 kg/d beginning at LD 2. The ration was gradually increased by 1 kg/d until d 6 postpartum, and then the  
85 sows were allowed *ad libitum* experimental diets and water. During LD, sows were fed daily at 06:30,  
86 10:00, 15:30, and 20:00. The sows were housed individually in GD crates (2.2 m length × 0.65 m width)  
87 and were transferred to individual farrowing crates (2.2 m length × 1.5 m width) at GD 110.

#### 88 Sample collection and laboratory procedures

89 The numbers of total piglets born, alive and stillborn were recorded on the farrowing day. Body  
90 length was measured from the occipital bone to the root of the tail. BW and length were used for the  
91 calculation of body mass index (BMI):  $[BW \text{ (kg)} / \text{length} \text{ (m)}^2]$  [19]. Cross-fostering was maintained  
92 within the diet treatment to adjust the small size to approximately  $12.20 \pm 0.21$  piglets per sow within 3  
93 d after birth. Cross-fostering occurred within the treatments as GD management of the sows was standard.  
94 Death rates were recorded after cross-fostering had been completed, and those dead piglets were not  
95 replaced. No feed was offered to the piglets, and sow troughs were sufficiently high to prevent the piglet

96 from eating sow feed. However, piglets were allowed ad libitum water via nipple drinkers at weaning,  
97 and the number of weaned piglets was recorded. Piglets were weighed within 24 h of birth (LD 0) and  
98 weighed on LD 3, 7, 14, and upon weaning. After weaning, estrus was assessed in sows for 21 d, and the  
99 weaning-to-estrus interval (WEI) of sows was recorded. Unconsumed feed was weighed daily, and the  
100 average daily feed intake (ADFI) was evaluated.

101 Blood samples (5 mL) were collected from all sows via jugular venipuncture 2 h after feeding on  
102 the morning of LD 1 and on the weaning day. Blood samples were collected from 12 randomly selected  
103 piglets per group on LD 19 via jugular venipuncture. Blood samples were centrifuged for 10 min at 1,100  
104 g force and 4 °C, and serum was collected and stored at -20 °C for further analysis. Colostrum samples  
105 (30 mL) were hand stripped from median mammary glands on both sides from all sows within 3 h of  
106 farrowing and were analyzed fresh. Milk yield was calculated as litter gain  $\times$  4.2 [20].

107 Every day during LD, we ranked the feces of each sow by visual qualitative evaluation. Feces were  
108 scored according to [21]: 0 = absence of feces, 1 = dry and pellet-shaped, 2 = between dry and normal,  
109 3 = normal and soft, but firm and well-formed, 4 = between normal and wet, still formed but not firm  
110 and 5 = very wet feces, unformed and liquid. Depending on the number of consecutive days with no fecal  
111 production, we classified the grade of constipation as mild (no feces for 2 consecutive days), severe (no  
112 feces for 3 or 4 consecutive days), and extremely severe (no feces for more than 5 consecutive days).

113 At LD 19, fresh fecal samples were collected in sterile 2-mL centrifuge tubes without any treatment,  
114 and these samples were stored at -80 °C until they were used for 16S rDNA gene sequencing analysis.  
115 Fecal samples were collected in plastic bags and fixed on site by mixing with 10% hydrochloric acid (10  
116 mL hydrochloric per 100-g fresh feces) on the last 3 days of LD. The total weight of feces was not less  
117 than 200 g per sow. Diet samples were collected in plastic bags by quartation at the same time. Fecal and

118 diet samples were stored at -20 °C before the apparent nutrient digestibility assessment.

### 119 **Chemical analyses**

120 Serum total protein (TP) and blood urea nitrogen (BUN) metabolite assays were conducted with  
121 commercial kits purchased from Nanjing Jiancheng Biotechnology Co., Ltd, China. The absorbance was  
122 measured using a microplate reader, and the amount of serum BUN was converted. Colostrum  
123 composition analysis was detected by the principle of the Fourier transform infrared technique using  
124 MilkoScan TM FT2 (Combifoss FT, FOSS Electric, Hillerød, Denmark). Fecal samples were thawed and  
125 over-dried at 65 °C for 72 h. Dried feces and experimental diets were ground and passed through a 1 mm  
126 screen before chemical analysis. Hydrochloric acid insoluble ash (AIA) was used as an indigestible  
127 marker to assess the apparent digestibility of the dietary components. The contents of AIA, dry matter  
128 (DM), CP, crude fiber (CF), ether extract (EE), calcium (Ca), and total phosphorus (P) were determined  
129 according to official methods of analysis [22]. The apparent total tract digestibility (ATTD) was  
130 determined using the following formula [23]:

$$131 \quad \text{ATTD (\%)} = 100 - [(AIA_D \div AIA_F) \times (DC_F \div DC_D) \times 100\%],$$

132 Where  $AIA_D$  indicates the AIA concentration in the diet;  $AIA_F$  indicates the AIA concentration in the  
133 feces;  $DC_F$  indicates the dietary component concentration in the feces, and  $DC_D$  indicates the dietary  
134 component concentration in the diet.

### 135 **Quantification of fecal bacteria**

136 Microbial genomic DNA was isolated from fecal samples using a QIAamp-DNA stool mini kit  
137 (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Serial dilutions of positive  
138 plasmids were saved in our laboratory. They were used to create standard curves using quantitative real-  
139 time PCR (Vazyme Biotech, USA) with species and genus-specific primers (Table 3), permitting



140 estimations of absolute quantification based on individual gene copies. The reactions were performed in  
141 a total volume of 20  $\mu\text{L}$ , containing 2- $\mu\text{L}$  of template DNA, 0.4- $\mu\text{L}$  of forward and reverse primers, 10-  
142  $\mu\text{L}$  of SYBR Green PCR Master Mix (Vazyme Biotech, USA), 0.4- $\mu\text{L}$  of Rox-2, and 6.8- $\mu\text{L}$  of nuclease-  
143 free water. The thermal cycling conditions involved an initial denaturation step at 95 °C for 30 s, followed  
144 by forty cycles of 95 °C for 10 s and the appropriate annealing temperature (Table 3) for 30 s. Then, a  
145 melting curve was produced to confirm the specificity of amplification. The data were generated as gene  
146 copy numbers per gram of wet feces.

#### 147 **Statistical analyses**

148 Data were analyzed using the SPSS 20.0 package (SPSS Inc., Chicago, IL, USA), and a one-way  
149 ANOVA was performed on the four treatment groups. One-way ANOVA also analyzed linear and  
150 quadratic polynomial trends. When the effect was significant, means were compared with each other  
151 using the Tukey multiple range test. Before analysis, the data were tested for normality and  
152 homoscedasticity. A sow and litter were treated as an experimental unit, and dietary treatment was the  
153 only fixed experimental effect. Data are presented as the means and SEM. Differences were considered  
154 significant when  $p < 0.05$ .

## 155 **RESULTS**

### 156 **Sow apparent nutrient digestibility**

157 The effects of FBW on the apparent digestibility of sows are shown in Table 4. The apparent digestibility  
158 of DM was significantly increased by FBW supplementation by treatment ( $p < 0.01$ ), linear ( $p < 0.01$ ),  
159 and quadratic analysis ( $p < 0.05$ ). Among them, the apparent digestibility of P was significantly higher  
160 with increasing concentrations of FBW by treatment ( $p < 0.01$ ), linear ( $p < 0.01$ ), and quadratic analysis  
161 ( $p < 0.01$ ). There was no significant effect on the apparent digestibility of CF, EE, and Ca.

162 **Sow and piglet performance**

163 As shown in Table 5, during the first week of LD, the ADFI of sows in the FBW 6 and FBW 9  
164 groups was significantly higher than that of the CON group ( $p < 0.01$ ). During the third week of LD, the  
165 ADFI was significantly increased by linear analysis ( $p < 0.05$ ). The diets supplemented with FBW  
166 significantly increased the ADFI during the LD period by treatment ( $p < 0.05$ ), linear ( $p < 0.05$ ), and  
167 quadratic analysis ( $p < 0.05$ ). Maternal FBW supplementation did not affect litter size or the weaning  
168 survival rate during the LD period. Piglets at birth, after cross-foster, and at LD 7 showed no treatment  
169 effects on litter weight. However, the litter weight of the CON group sows was significantly lighter than  
170 that of the other 3 groups at LD 14 by treatment ( $p < 0.05$ ), linear ( $p < 0.05$ ), and quadratic analysis ( $p <$   
171  $0.05$ ). The litter weight of the CON group sows was significantly lighter than that of the other 3 groups  
172 at LD 19 by treatment ( $p < 0.01$ ), linear ( $p < 0.01$ ), and quadratic analysis ( $p < 0.05$ ). The mean piglet  
173 weight and piglet average daily gain were not affected by maternal FBW supplementation throughout  
174 the LD period. The milk yield of the treatment groups was significantly increased ( $p < 0.05$ ). The BMI  
175 of the treatment groups at birth was found by quadratic analysis to be significantly increased compared  
176 with that of the CON group by quadratic analysis ( $p < 0.05$ ).

177 **Chemical analyses**

178 As shown in Table 6, maternal FBW supplementation did not affect the CP, lactose, fat, solid, or  
179 solid-not-fat contents. A significant decrease was observed in urea concentration ( $p < 0.05$ ). As shown in  
180 Table 7, the concentration of BUN in sows and piglets' serum at weaning was found to be significantly  
181 decreased ( $p < 0.01$ ), and the concentration of TP in piglet serum at weaning was significantly increased  
182 in FBW groups compared with that of the CON group ( $p < 0.01$ ). The concentration of BUN in weaning  
183 sows and piglet serum was significantly different among the treatments ( $p < 0.01$ )

184 **Fecal scores, bacterial counts, and constipation for sows**

185 As shown in Table 8, compared with the CON group, sows treated with FBW had lower fecal  
186 *Escherichia coli* counts by treatment ( $p < 0.01$ ), linear ( $p < 0.05$ ) and quadratic analysis ( $p < 0.05$ ). The  
187 fecal count of *Clostridium cluster XIVa* in the FBW 3 group was significantly decreased compared to that  
188 in the CON group ( $p < 0.05$ ). As shown in Fig. 1A, during the 3 weeks of LD, the fecal scores of the  
189 FBW 6% and FBW 9% groups were significantly higher ( $p < 0.05$ ) than the fecal scores of the CON  
190 group every week, and the fecal scores of the FBW 3% group were significantly higher ( $p < 0.05$ ) than  
191 the fecal scores of the CON group only in the second week. The grade of constipation for the sows is  
192 shown in Fig. 1B.

193 **DISCUSSION**

194 **Sow apparent nutrient digestibility**

195 In the current study, DM and P retention were higher in the FBW treatments than in the CON  
196 treatments. Studies assessing the effects of FBW in sows are limited. The function and morphology of  
197 the small intestine are often used to measure indicators of digestion and nutrient absorption. Studies have  
198 shown the utilization of supplemented fermented diets to modulate the intestinal microbial community  
199 structure and activity and enhance the integrity and function of the intestinal epithelial barrier [24-27].  
200 In addition, a study indicated that fermented soya bean extracts reduce the adhesion of enterotoxigenic  
201 *E. coli* to intestinal epithelial cells in pigs and prevent diarrheal diseases [28]. Although the small intestine  
202 morphology was not investigated in this study, the positive effect of fermented forage on intestinal  
203 function may lead to the enhancement of nutrient digestion and absorption ability. Since a large amount  
204 of P is often produced in fermented feed [29,30], it is not surprising that a high preservation rate of P was  
205 observed in the results.

206 **Sow and piglet performance**

207 Growth performance is an important indicator of the quality of feed fermentation. We observed a  
208 significant increase in the ADFI in FBW-treated sows during LD. Several studies have reported that  
209 fermented feed could enhance the growth performance of the herd. Sows' ADFI and litter weight  
210 increased when LD diets were supplemented with fermented corn and soybean meal mixed feed [31].  
211 However, Wang et al. observed that when 5% fermented soybean meal was added to the basal diet from  
212 GD 85 to weaning, the weaning litter weight and mean BW were not affected [32]. Various potential  
213 factors might result in these discrepancies. One factor is the ingredients in the supplementation. Wang et  
214 al. used soybean meal as fermented feed, whereas the products used in our study were mixed, including  
215 bean dregs and wheat bran. Another factor was the difference in probiotics. We used *L. acidophilus*, *S.*  
216 *cerevisiae*, *B. subtilis*, and *E. faecalis* to produce fermented feed in the present study. In contrast, Wang  
217 et al. used *B. subtilis*, *Hansenula anomala* and *Lactobacillus casei* [32]. Different probiotic combinations  
218 can produce different proteases that can influence the absorption and digestion of nutrients. This may  
219 improve the overall protease activity of the fermented feed, promote the growth of fermentation  
220 microorganisms, improve the content of organic acids, reduce pH, inhibit the growth of harmful  
221 microorganisms and improve the quality of FBW [33, 34]. Furthermore, the number and weight of piglets  
222 after cross-sending may also affect the piglet weaning weight. In addition, we observed that the 9% FBW-  
223 treated sow ADFI was lower between LD8-LD14 and LD15-LD19 than the 6% FBW-treated sow ADFI  
224 according to quadratic analysis. This may be because too much insoluble fiber can shorten the residence  
225 time of the chyme in the intestine, and because too much soluble fiber can adhere to the surface of the  
226 chyme to form a nutrient barrier, which is unfavorable for the digestion of nutrients, in turn, affecting the  
227 appetite of sows [35].

228 **Colostrum composition, total serum protein, and blood urea nitrogen concentrations**

229 The quality of colostrum and the growth of newborn piglets largely depend on the nutrient intake  
230 and utilization of sows. In the current study, the decreased urea content in colostrum reflects the increased  
231 nitrogen utilization in sows. The growth performance and health conditions of newborn piglets mainly  
232 depend on the quality and quantity of colostrum and milk from sows. Therefore, the elevated litter weight  
233 may have resulted from elevated milk yield. Nutrient utilization, especially energy and protein absorption,  
234 during LD affects the milk yield of sows [36]. These results showed that the sows supplemented with  
235 FBW absorbed more energy and protein than sows in the CON treatment by increasing the sows' ADFI  
236 and apparent digestibility; then, the sows produced more milk for the piglets.

237 BUN, a waste byproduct of protein breakdown, is an indicator used to assess amino acid balance  
238 and protein metabolism status. A good balance of amino acids in the diet could reduce the content of  
239 BUN [37]. In the present study, supplementation with FBW reduced the serum BUN concentration in  
240 sows and piglets on the weaning day, demonstrating that supplementation with FBW could improve the  
241 efficiency of protein utilization in sows. The significant change in the content of urea in colostrum also  
242 confirms this. This may be due to the high content of acid-soluble protein in the FBW, which animals  
243 more easily absorb. This finding was consistent with the TP status in piglets on the weaning day, in which  
244 supplementation with FBW significantly increased the concentration of serum TP in piglets on the  
245 weaning day. However, this study showed that the treatment sows had a significantly lower TP content  
246 than CON sows on the weaning day. We speculate that FBW-treated sows consumed more energy and  
247 protein to produce milk. The serum TP and BUN concentrations in sows at birth were not significantly  
248 affected by FBW supplementation. The duration of supplementation may determine its influence.

249 **Fecal scores and bacterial counts for sows**

250 The fiber content in the diets greatly affected the intestinal activity of sows after farrowing. In the  
251 present study, the results showed that supplementation with FBW helped the intestine to avoid extended  
252 constipation. This may be due to the fiber from wheat bran and bean dregs in the treatment group. In the  
253 three weeks of LD, the sows in the treatment group always had higher fecal scores than the CON group.  
254 The high fecal score values indicate that the intestine was more active during LD. The study reported  
255 that a high-fiber diet could decrease extended constipation during the perinatal period by promoting  
256 intestinal activity [38]. The fecal scores of sows were lowest at birth and rose gradually until the end of  
257 this experiment. The sows in the treatment group recovered good intestinal activity sooner than those in  
258 the CON group.

259 FBW significantly reduced fecal *E. coli* counts in all treatment groups. *E. coli* is a necessary factor  
260 for healthy intestinal microflora of sows and contains many pathotypes that lead to various diseases. The  
261 decrease in *E. coli* could reduce the disease risk of sows. Meanwhile, elevated levels of *E. coli* and  
262 *Clostridium* were found in the intestine of constipated patients [39, 40]. A reduction of *Clostridium*  
263 *enterica* improves constipation symptoms [41-43]. The high content of fiber and live bacteria in FBW  
264 may be responsible for changing the flora in sow feces. The decrease in *E. coli* and *Clostridium* may  
265 have contributed to stimulating intestinal activity [44], which promotes better physical condition and  
266 production performance of lactating sows.

## 267 CONCLUSIONS

268 This study demonstrated that dietary supplementation with FBW during late GD and LD increased  
269 ADFI and protein utilization and attenuated constipation in sows, which increased milk yield and piglet  
270 growth performance. FBW also improved fecal scores and decreased the content of pathogenic bacteria  
271 in feces. These findings suggest that supplementation with FBW helped improve sow production

272 performance, and 6% FBW is recommended as a suitable dose for the best product performance in sows

273 and piglets.

274 **Competing interests**

275 No potential conflict of interest relevant to this article was reported.

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281 **Authors' contributions**

282 Conceptualization: Li CM.

283 Data curation: Liu JZ, Zhao LY.

284 Formal analysis: Liu JZ, Zhao LY, Li ZJ.

285 Methodology: Liu JZ, Zhao LY, Li ZJ, Li YS.

286 Validation: Liu JZ, Zhao LY.

287 Investigation: Li CM, Li YS.

288 Writing - original draft: Liu JZ.

289 Writing - review & editing: Wang K.

290

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408 **Figure legend**

409 Fig.1. Effect of fermented mixture of bean dregs and wheat bran (FBW) supplementation on average  
410 qualitative fecal scores (A), grade of constipation (B) of sows during lactation. Score 0=absence of feces,  
411 1=dry and pellet-shaped, 2=between dry and normal, 3=normal and soft, but firm and well-formed,  
412 4=between normal and wet, still formed but not firm and 5=very wet feces, unformed and liquid.  
413 Classification of the grade of constipation: mild (no feces for two consecutive days), severe (no feces for  
414 three or four consecutive days), and extremely severe (no feces for more than five consecutive days).  
415 Values are means  $\pm$  SEM. The asterisk indicates the degree of significance compared to control group (\*  
416 =  $p < 0.05$ ; \*\* =  $p < 0.01$ ).

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425 **Table 1** Nutrient composition of fermented mixture of bean dregs and wheat bran (FBW).

Items	Bean dregs and wheat bran	FBW (wet)	FBW (dry basis)
Moisture content, %	45.83	40.85	11.63
Crude protein, %	10.89	12.56	19.40
Acid soluble protein, %	1.55	2.91	5.53
Crude fiber, %	5.12	4.83	7.06
Neutral detergent fiber, %	31.32	27.10	42.96
Acid detergent fiber, %	9.52	7.95	12.76
pH value	5.57	4.75	4.86

426

427 **Table 2** Ingredients and nutrient composition of fermented mixture of bean dregs and wheat bran

428 (FBW), % (as-fed).

Items	Gestation diet				Lactation diet			
	CON	FBW	FBW	FBW	CON	FBW	FBW	FBW
	0	3	6	9	0	3	6	9
Ingredients								
Corn	36.84	33.87	30.9	27.93	38.30	35.33	32.36	29.39
Barley	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Wheat germ	12.90	12.90	12.90	12.90	6.30	6.30	6.30	6.30
Soybean oil	1.20	1.87	2.54	3.21	2.80	3.47	4.14	4.81
Soybean meal	15.50	14.80	14.10	13.40	18.20	17.5	16.8	16.1
FBW	0.00	3.00	6.00	9.00	0.00	3.00	6.00	9.00
Soy hulls	7.00	7.00	7.00	7.00	6.00	6.00	6.00	6.00
Fish meal (65%)					1.00	1.00	1.00	1.00
Brown sugar					1.00	1.00	1.00	1.00
Limestone	1.23	1.23	1.23	1.23	1.22	1.22	1.22	1.22
Calcium hydrogen phosphate	1.52	1.52	1.52	1.52	1.59	1.59	1.59	1.59
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Fluid methionine (88%)	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10
Premix <sup>2</sup>	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Rice bran meal (carrier)	0.61	0.61	0.61	0.61	0.46	0.46	0.46	0.46
Salt	0.50	0.50	0.50	0.50	0.49	0.49	0.49	0.49
Sodium bicarbonate	0.45	0.45	0.45	0.45				
Potassium chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mycotoxin adsorbent	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L-lysine (98%)	0.07	0.07	0.07	0.07	0.37	0.37	0.37	0.37
L-threonine (98.5%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

L-tryptophan (20%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Valine	0.04	0.04	0.04	0.04	0.14	0.14	0.14	0.14
Nutrient composition <sup>2</sup>								
ME, Mcal/kg	3.20	3.20	3.20	3.20	3.30	3.30	3.30	3.30
Dry matter	87.69	87.90	87.87	87.65	88.16	88.34	87.67	88.03
Crude protein	16.39	16.48	16.47	16.40	16.98	17.27	16.85	17.09
Ether extract	4.06	5.10	6.12	7.14	5.17	6.21	7.22	8.23
Ash	6.21	6.28	6.40	6.49	5.98	6.09	6.24	6.27
Total phosphorus	0.65	0.73	0.76	0.70	0.62	0.58	0.65	0.61
Calcium	0.84	0.84	0.86	0.86	0.83	0.85	0.85	0.83
Methionine	0.32	0.32	0.31	0.31	0.31	0.31	0.30	0.31
Lysine	0.90	0.93	0.93	0.91	0.99	1.04	1.05	1.02

429 <sup>1</sup>Premix provided the following per kg of complete diet: vitamin A, 50KIU; vitamin D3, 15KIU; vitamin  
430 E, 200IU; vitamin K3, 8mg; vitamin B1, 10mg; riboflavin, 12mg; Vitamin B6, 12mg; niacin, 200mg;  
431 folic acid, 6mg; pantothenic acid, 100mg; sodium chloride, 3.0-6.0%; choline chloride, 2000mg; iron,  
432 1200mg; copper, 200mg; manganese, 120mg; zinc, 800 mg; iodine, 1.4mg; selenium, 1.0mg; Ca, 4.0-  
433 8.0%; total P, 1.5%; Lysine, 0.16%.

434 <sup>2</sup>ME were a calculated valued, while the others were measured values.

435

436 **Table 3** Species and genus specific primers used for real time PCR to profile selected bacteria.

Target organisms	Primers	Sequence (5'-3')	Product size, bp	Annealing temperature, °C	Reference
<i>Total bacteria</i>	<i>Total-F</i>	GTGSTGCAYGGYYGTCGTCA	123	60	(Suzuki et al., 2000)
	<i>Total-R</i>	ACGTCRTCCMCNCCTTCCTC			
<i>Clostridium cluster IV</i>	<i>C.leptum-F</i>	GCACAAGCAGTGGAGT	240	60	(Matsuki et al., 2004)
	<i>C.leptum-R</i>	CTTCCTCCGTTTTGTCAA			
<i>Clostridium cluster XIVa</i>	<i>Clo14-F</i>	CGGTACCTGACTAAGAAGC	189	60	(Matsuki et al., 2004)
	<i>Clo14-R</i>	AGTTTTYATTCTTGCGAACG			
<i>Lactobacillus</i>	<i>Lac-F</i>	AGCAGTAGGGAATCTTCCA	341	64	(Khafipour et al., 2009)
	<i>Lac-R</i>	ATTCCACCGCTACACATG			
<i>Bacteroides spp</i>	<i>Bac303-F</i>	GAAGGTCCCCCACATTG	126	60	(Bartosch et al., 2004)
	<i>Bfr-Fmrev-R</i>	CGCKACTTGGCTGGTTCAG			
<i>Escherichia coli</i>	<i>E.coli-F</i>	CATGCCGCGTGTATGAAGAA	95	60.8	(Huijsdens et al., 2002)
	<i>E.coli-R</i>	CGGGTAACGTCAATGAGCAAA			

437



438 **Table 4** Effects of fermented mixture of bean dregs and wheat bran (FBW) supplementation on  
 439 apparent nutrient digestibility, %.

Treatment	CON	FBW 3	FBW 6	FBW 9	SEM	<i>p</i> -value		
	FBW inclusion (%)	0	3	6		9	Treatment	Linear
DM	80.81 <sup>b</sup>	83.30 <sup>a</sup>	83.61 <sup>a</sup>	83.47 <sup>a</sup>	0.29	< 0.01	< 0.01	0.034
CP	80.80	81.58	82.89	82.98	0.35	0.100	0.017	0.624
CF	62.39	60.61	58.58	53.26	0.88	0.302	0.072	0.616
EE	76.11	76.82	75.90	79.29	0.65	0.265	0.155	0.317
P	52.30 <sup>c</sup>	63.53 <sup>b</sup>	63.88 <sup>b</sup>	66.94 <sup>a</sup>	0.96	< 0.01	< 0.01	0.001
Ca	56.47	58.86	59.88	60.66	1.22	0.691	0.239	0.759

440 <sup>a, b</sup> Within a row, means without a common lowercase superscript differ ( $p < 0.05$ ).

441 Abbreviations: DM: dry matter; CP: crude protein; CF: crude fiber; EE: ether extract.

442

**Table 5** Effect of fermented mixture of bean dregs and wheat bran (FBW) supplementation on reproductive performance in piglets.

Treatment	CON	FBW3	FBW6	FBW9	SEM	<i>p</i> -value		
	0	3	6	9		Treatment	Linear	Quadratic
ADFI in lactation, Kg								
LD 1-LD 7	3.50 <sup>b</sup>	3.75 <sup>ab</sup>	3.86 <sup>a</sup>	3.93 <sup>a</sup>	0.04	0.006	0.498	0.772
LD 8-LD 14	5.66	6.38	6.33	6.20	0.12	0.119	0.131	0.071
LD 15-LD 19	6.10 <sup>c</sup>	6.49 <sup>b</sup>	7.00 <sup>a</sup>	6.75 <sup>a</sup>	0.14	0.100	0.040	0.211
LD 1- LD 19	4.98 <sup>b</sup>	5.44 <sup>a</sup>	5.60 <sup>a</sup>	5.51 <sup>a</sup>	0.084	0.031	0.014	0.017
Litter size, No/litter								
Total born	14.67	13.83	12.33	15.33	0.59	0.329	0.925	0.118
Born alive	13.50	13.33	10.33	13.67	0.60	0.154	0.629	0.140
Still born	1.17	0.50	2.00	1.67	0.47	0.715	0.495	0.865
LD 3	11.67	13.00	11.33	12.25	0.26	0.085	0.871	0.424
LD 19	11.00	12.40	10.90	11.75	0.29	0.122	0.962	0.766
Weaning survival rate, %	94.44	95.49	95.89	95.83	1.31	0.788	0.729	0.901
Litter weight, Kg								
LD 1	16.79	16.56	16.86	20.59	0.68	0.115	0.057	0.139
LD 3	20.64	21.59	23.50	22.48	0.85	0.710	0.351	0.621
LD 7	28.11	32.83	34.69	31.63	1.15	0.215	0.190	0.099
LD 14	43.50 <sup>b</sup>	48.72 <sup>a</sup>	48.01 <sup>a</sup>	47.14 <sup>a</sup>	1.43	0.042	0.034	0.047
LD 19	59.17 <sup>b</sup>	70.35 <sup>a</sup>	65.61 <sup>a</sup>	69.42 <sup>a</sup>	2.35	< 0.01	< 0.01	0.045
Piglet mean weight, Kg								
LD 1	1.26	1.27	1.44	1.36	0.05	0.604	0.329	0.705
LD 3	1.78	1.63	1.79	1.79	0.08	0.880	0.820	0.640
LD 7	2.37	2.42	2.64	2.52	0.11	0.847	0.520	0.713
LD 14	3.81	4.09	4.25	4.14	0.16	0.822	0.462	0.567
LD 19	5.21	5.35	6.01	5.38	0.42	0.974	0.675	0.708

Piglet ADG, g/d								
LD 1-7	183.60	216.80	233.80	194.80	8.72	0.172	0.498	0.042
LD 8-14	205.17	238.80	254.20	236.40	10.00	0.360	0.204	0.215
LD 15-19	276.00	261.80	274.80	257.80	17.94	0.982	0.947	0.747
LD 3-19	207.40	240.60	255.60	232.80	8.80	0.281	0.250	0.121
Milk yield <sup>1</sup> , Kg	163.63 <sup>b</sup>	204.80 <sup>a</sup>	196.06 <sup>a</sup>	197.13 <sup>a</sup>	6.06	0.041	0.033	0.046
WEI	5.00	4.33	5.17	4.50	0.19	0.344	0.790	0.924
BMI, Kg/m <sup>2</sup>	20.08	23.87	23.03	22.14	0.55	0.081	0.253	0.031

Cross-fostering was kept within the diet treatment in 3 d after birth, the data of LD 3 was recorded after Cross-fostering.

<sup>1</sup>Milk yield = litter gain × 4.2.

<sup>a, b</sup> Within a row, means without a common lowercase superscript differ ( $p < 0.05$ ).

Abbreviations: LD: lactation day. ADFI: average daily feed intake; ADG: average daily gain; WEI: weaning-to-estrus interval; BMI: body mass index of new born piglets.

**Table 6** Effect of fermented mixture of bean dregs and wheat bran (FBW) supplementation on colostrum composition.

Treatment	CON	FBW 3	FBW 6	FBW 9	SEM	<i>p</i> -value		
	0	3	6	9		Treatment	Linear	Quadratic
Protein, %	17.58	17.08	16.63	16.99	0.54	0.949	0.669	0.711
Lactose, %	3.69	3.77	3.34	3.55	0.10	0.487	0.374	0.750
Fat, %	4.8	4.93	4.65	4.68	0.18	0.953	0.706	0.905
Total solid, %	29.92	28.87	27.60	28.42	0.70	0.722	0.383	0.526
Urea, mg/mL	78.37 <sup>a</sup>	76.89 <sup>ab</sup>	70.44 <sup>ab</sup>	67.32 <sup>b</sup>	2.86	0.031	0.005	0.764
Solids-not-fat, %	24.81	23.14	22.17	22.84	0.56	0.405	0.181	0.307

<sup>a, b</sup> Within a row, means without a common lowercase superscript differ ( $p < 0.05$ ).

**Table 7** Effect of fermented mixture of bean dregs and wheat bran (FBW) supplementation on blood metabolites of sows and piglets.

Treatment	CON	FBW 3	FBW 6	FBW 9	SEM	<i>p</i> -value		
	0	3	6	9		Treatment	Linear	Quadratic
TP level of sows, g/L								
LD 1	78.94	77.40	77.63	72.30	2.42	0.824	0.412	0.721
LD 19	76.53	69.76	76.43	74.14	1.14	0.109	0.053	0.027
BUN level of sows, mmol/L								
LD 1	4.97	4.22	4.15	3.88	0.20	0.249	0.070	0.487
LD 19	6.82 <sup>a</sup>	5.93 <sup>b</sup>	5.65 <sup>b</sup>	5.78 <sup>b</sup>	0.13	< 0.01	< 0.01	0.024
TP level of piglets, g/L								
LD19	43.48 <sup>b</sup>	47.75 <sup>a</sup>	50.02 <sup>a</sup>	49.06 <sup>a</sup>	0.79	0.037	0.008	0.073
BUN level of piglets, mmol/L								
LD 19	3.72 <sup>a</sup>	2.66 <sup>b</sup>	2.91 <sup>b</sup>	3.16 <sup>ab</sup>	0.11	< 0.01	0.074	< 0.01

<sup>a, b</sup> Within a row, means without a common lowercase superscript differ ( $p < 0.05$ ).

Abbreviations: LD: lactation day. TP: Serum total protein. BUN: Blood urea nitrogen.

**Table 8** Effect of fermented mixture of bean dregs and wheat bran (FBW) supplementation on fecal flora of sows at weaning, log<sub>10</sub> CFU/g feces

Treatment	CON	FBW3	FBW6	FBW9	SEM	p-value		
	0	3	6	9		Treatment	Linear	Quadratic
<i>Total bacteria</i>	12.69	12.25	12.51	12.40	0.07	0.110	0.289	0.208
<i>Escherichia coli</i>	10.01 <sup>a</sup>	8.27 <sup>b</sup>	8.80 <sup>b</sup>	8.76 <sup>b</sup>	0.19	< 0.01	0.029	0.014
<i>Lactobacillus</i>	11.57	11.06	11.08	10.01	0.35	0.465	0.153	0.670
<i>Bacteroides spp</i>	11.99	11.63	11.78	11.66	0.05	0.090	0.076	0.293
<i>Clostridium cluster IV</i>	10.66	10.17	10.48	10.30	0.08	0.172	0.292	0.402
<i>Clostridium cluster XIVa</i>	10.42 <sup>a</sup>	9.56 <sup>b</sup>	9.94 <sup>ab</sup>	9.91 <sup>ab</sup>	0.10	0.030	0.130	0.051

<sup>a, b</sup> Within a row, means without a common lowercase superscript differ ( $p < 0.05$ )