Original Paper

Effect of fermented botanical product on weaning stress in piglets

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Abstract This study aimed to evaluate the effect of dietary supplementation with a fermented botanical product (FBP) on weaning stress in piglets. Effects were measured in terms of fecal consistency and cortisol levels in the coat as a marker of stress. Fecal consistency was observed daily in the afternoon and recorded on a 5-point scale and cortisol levels were determined at the following 3 times: on day 21, at the start of supplementation to assess change in the diet; on day 50, 1week after moving the piglets to the fattening pen, to assess adaptation to a new environment; and on day 81, the final day of experiment. No significant difference in fecal consistency scores were observed; however, an increase in wet and soft normal feces was noted in the experimental group, possibly due to the change in intestinal conditions induced by the FBP. The cortisol level in the coat of the experimental group continued to decrease during the period, while that of the control group leveled off. These findings suggest that the FBP contains useful fermented substances that directly and indirectly affect stress levels in piglets.

Key words: fermented botanical product, intestinal health, cortisol level

Receipt of Ms: 14.12.2021. Accepted: 31.01.2022.

Journal of Japanese Animal Production Environment Science No.21(1) pp14–20. 2022

Introduction

The primary goal of nursery piglet management is smooth weaning in order to avoid delayed growth and diseases. Accordingly, the weaning period is considered one of the most critical periods in pig management. A number of stress factors, including removal from the sow, dietary changes, and adaptation to a new environment can negatively affect the immune system and lead to intestinal dysfunction during weaning. These stress factors are also associated with the onset of diseases during the postweaning stage as well as diarrhea, dehydration, growth retardation, and in severe cases, sudden death [1]. Postweaning diarrhea tends to be associated with the proliferation of *Escherichia.coli* and oral treatment with antibiotics is widely used in pig production. However, elimination of in-feed antibiotics has become a recent focal point. The physiological roles of in-feed antibiotics are to regulate unfavorable bacteria and encourage favorable intestinal conditions. Accordingly, they are widely used by almost all pig farmers in Japan. However, antibiotic resistance has become a severe problem, highlighting the need for alternative solutions. In line with this supplementation with fermented feed was previously found to support intestinal health [4,5] by modifying the protein amino

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acid profile [8] and/or reducing the raw fiber content [6]. Fermented substances are also known to induce changes to the composition of intestinal bacteria. In our previous studies [13,15], we revealed that supplementation with a fermented botanical product (FBP) contributed to a reduction in *E.coli* in the gut of fattening pigs. Furthermore fecal consistency is changed in accordance with the various stress during the nursing and weaning period. The purpose of the present study was to evaluate the effect of dietary supplementation with FBP on fecal consistency and cortisol level in the coat as a marker of stress. Chronological changes in cortisol level during the post-weaning stage were also investigated.

Materials and methods

1. Fermented botanical product (FBP)

The FBP used in this study was established over a period of 3.25 years by Manda Fermentation Co. Ltd. (Onomichi, Japan). Brown sugar, rice, apples, oranges, bananas, persimmons, pineapples, soybeans, carrots, seagrass, grapes, honey, garlic, sesame, and bayberries were fermented with *Lactobacillus* and *Saccharomyces* at room temperature and the resulting paste was mixed with rice bran for use as a dietary supplement.

2. Animal and management

Experimental feeding was conducted at a personal pig farm in Makinohara, Japan using eight litters of crossbred (WLD: Large White, Landrace, and Duroc) pigs. Eight litter was born at the same time and included the same number of piglets. The piglets were divided into two groups (control and experimental) consisting of groups from each litter. Basal management and feed composition were in accordance with standard practices of the cooperative farm. Growth performance was evaluated by daily observation based on the farmer's experience because it was not possible to determine actual body weight on this farm. The control group was fed commercial artificial milk as a pre-starter and starter while the experimental group was given the same starter plus 0.125% FBP (Table 1). Experimental feeding was carried out from day 21 after birth until day 81 (Fig.1).

The main components of the pre-starter were dried whey, dried skim milk, fish meal, plasma protein concentrate, wheat feed flour, maize, bread crumbs, dehulled soybean meal, fermented dehulled soybean meal, and defatted rice bran. The main components of the starter were maize, dry-heat dehulled soybean, wheat feed flour, dehulled soybean meal, fish meal, dried whey, and dried skim milk. The piglets were housed in farrowing pens equipped with a polyvinyl-coated wire floor during the nursing period, and in growing pens equipped with a stainless-steel floor during group feeding. The area of the pens was

Table 1. Compositions of the pre-starter and starter (%)

Pre-starter/first stage of weaning	Control	Experiment
Artificial milk	97.8	97.8
Defatted rice bran	0.625	-
Defatted rice bran plus FBPs	-	0.625
Viable fungus	1.4	1.4
Antibiotics	0.2	0.2
Starter/second stage of weaning		
Artificial milk producted by grains	99.6	99
Defatted rice bran plus FBPs	-	0.625
Viable fungus	0.2	0.2
Antibiotics	0.2	0.2

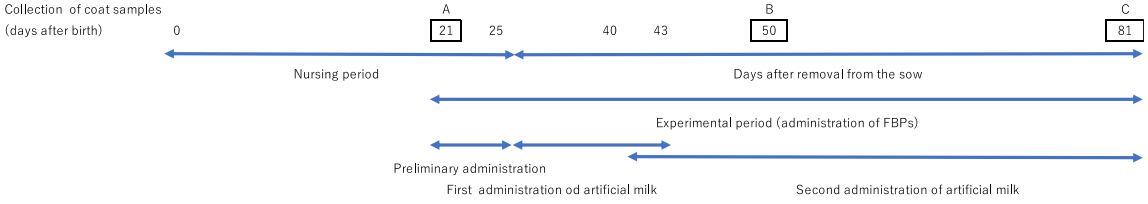

Figure 1. Experimental design of this study

4.05m^2 and 2.70m^2, respectively. They contained no air conditioning, and the field temperature ranged from 0.5°C and 35.6°C during the experimental period of October to February. Each pen was fitted with an infrared heater, and the piglets were given access to feed and water *ad libitum*.

Fecal consistency was observed daily in the afternoon and recorded on a 5-point scale as shown in Table 2 and Figure 2. Coat samples for the analysis of cortisol were obtained three times as follows: on day 21, at the start of starter supplementation to assess the changes in diet: on day 50, 1week after moving the piglets to the fattening pen to assess adaptation to a new environment; and on day 81, at the end of the experiment. About 1 g of coat sample was clipped on a surface of piglet's back under the mild pick up. Cortisol levels were determined by Dr. Hideaki Hayashi of Rakuno Gakuen University, and preliminary treatment was carried out by Dr. Takuji Hirayama of Ishikawa Prefectural University in accordance with a previous report [11].

The study was approved by the Animal Care and Use Committee of Manda Fermentation Co. Ltd..

3. Statistical analysis

Mean values (± SD of each replication) were evaluated by one-way analysis of variance with significance set at $P<0.05$.

Table 2. The 5-point scoring system used to determine fecal consistency

Score	Descriptiion
0	Hard and dryed (costipation)
1	Soft and wet condition (normal)
2	Mild diarrhea
3	Diarrhea
4	Srrong diarrhea

Figure 2. Feces appearance based on the 5-point scoring system.

Results and discussion

1. Fecal consistency

It was previously suggested that fermented food includes a number of useful microbiota that might improve fecal consistency as well as diarrhea and/or constipation in weaning piglets [9,10]. Fecal consistency scores obtained in this study are shown in Table 3. No significant differences were observed between the treatment and control groups. In the experimental group, an increase in wet and soft type feces was observed possibly due to changes in the intestinal conditions induced by variation in gut microorganisms following the administration of FBP, which would be consistent with a previous study [2]. We were unable to remove antibiotics from the piglets' diet, because management of post-weaning diarrhea is considered vital, and thus the lack of diarrhea may have been induced by the in-feed antibiotics. Prevention of diarrhea is the focus of piglet management, and fecal consistency is a standard observation point. However in this study, there were no obvious difference in fecal consistency, between groups. Fermented food was previously found to affect constipation with no effect on normal fecal consistency [8]. The results of the present study therefore suggest that the FBP used in this study played a role in improving the intestinal conditions of the weaning piglets.

2. Variation in cortisol levels in the coat

Cortisol levels are a well-known marker of stress: however, elevated levels in the blood are also

Table 3. Results of feces scores (%)

	Control			Experiment		
	Hard and dry	Soft and wet	Diarrhea	Hard and dry	Soft and wet	Diarrhea
The 1st try	98.6	1.4	0	97.2	2.8	0
The 2nd try	94.6	5.4	0	92.9	7.1	0
The 3rd try	90.9	9.1	0	87.3	12.7	0
Mean	95.1	4.9	0	92.9	7.1	0

Table 4. Cortisol levels in the coat (pg/mg)

Sample	Control	Experiment
A	6.42 ± 0.64	
B	1.46 ± 0.15	2.00 ± 0.26[a]
C	1.68 ± 0.20	1.22 ± 0.12[b]

Mean ± SE, n=30 (without timing A, n=60)

Different superscript letters represent a significant difference at $P<0.05$.

Samples A,B and C are collected in different timing in Figure 1.

representative of increased fat and/or sugar metabolism. Both physical and mental stress lead to increased blood cortisol levels, indicating the adaptation of the body. However, blood cortisol level, which shows the adaptation with acute response inducing the restriction for the blood collections, is not adapted in assessment of environmental condition. In contrast, levels of cortisol in the coat do not show acute changes, and are therefore useful for the analyzing environmental access. The cortisol level of the experimental group continued to decrease during this period, while that of the control group leveled off, but the difference between the groups was not significant (Table 4). Kim et al. [13] previously reported that supplementation with fermented soybean meal not only improved growth performance, feed intake, and overall health during the preweaning period, but also alleviated stress responses as indicated by reductions in stress hormone in Holstein calves after weaning. Moreover, Hannah et al. [12] reported that a *Saccharomyces cerevisiae* fermentation product mitigate cellular stress following a bout of single, prolonged submaximal exercise in young horses. Furthermore, Oh et al. [11] suggested that the protective effects of fermented glycated milk casein (FGc), which targets intestinal microbiota, is consistent with the important role of FGc in regulating the stress-related gut-brain axis. Thus, although the type of fermented food differs between this study and the above-mentioned reports, it is commonly agreed that the process of microbial fermentation converts food substrates into more nutritionally and functionally available products. Therefore the result of this study suggest that the fermented substances in the FBP have a positive effect on stress levels, acting both directly and indirectly.

3. Future prospects

Cortisol levels in the coat show that values were highest during the nursing period in both groups. Cortisol is synthesized via acetyl coenzyme A, which is derived from the active metabolism of sugar and fat. In line with this, the content and type of carbohydrates in milk and/or food were found to have a significant effect on the endocrine synthesis of cortisol [15]. This might explain why cortisol levels were highest during nursing. However, metabolism should subsequently

maintain these heigh levels after weaning when the piglets are fed pre-starter made with artificial milk and defatted rice bran, but in the case, a dramatic decrease was observed. These findings suggest that the variation in cortisol levels reflects hunger stress during nursing possibly due to the aggressive nature of feeding and the inadequate volume of milk. This is in contrast with the earlier suggestion, that weaning is the greatest stress factor during piglet management, and is consistent with the hypothesis that breastfeeding causes considerably more stress than formula feeding in human babies [7,14]. Adaptive creep feeding in piglets has been suggested. To optimize piglet management, assessment of the effect of different feeding conditions on cortisol levels during the nursing period is required, because only one such trial has never been conducted in Japan to date [16].

Acknowledgements

We would like to thank Dr. H. Hayashi, Dr. T. Hirayama, and the members of the cooperative farm for their valuable help during this study.

This report was presented as part of the proceedings of the 114th Japan Swine Science Meeting and the 19th Japan Animal Production Environment Science Meeting.

References

[1] Chen, M., X.Ye, D.Shen, C.Ma (2019) Modulatory effects of gut microbiota on constipation: The commercial beverage Yakult shapes stool consistency. J.Nurogastroenteral. Motil.: 25: 475-477. doi.org/10.5056/jnm19048.

[2] Czech,A., E.R.Grela, M.Kiesz (2021) Dietary fermented repeseed or/and soybean meal additives on performance and intestinal health of piglets. Scientific Reports: 11: 16952-16961. doi:org/ 10. 1038/s41598-021-96117-w.

[3] Gao,J., J.Yin, K.Xu, T.Li, Y.Yin (2019) What is the impact of diet on nutritional diarrhea associated with gut microbiota in weaning piglets: a system review. BioMed Res.Int. doi.org/10.1155/2019 /6916189.

[4] Hannah,C.V., J.L.Leatherwood, R.E.Martinez, S.A.Norton, S.H.White-Springer (2021) Dietary supplementation of a Saccharomyces cerevisiae fermentation product attenuates exercise-induced stress markers in young horses. J.Anim.Sci.: 99: 1-11. doi.org/10.1093/jas/skab199.

[5] Kim,M.H., C.H.Yun, C,H.Lee, J.K.Ha (2012) The effects of fermented soybean meal on immunophysiological and stress-related parameters in Holstein calves after weaning. J.Dairy Sci.: 95: 5203-5212. doi.org/10.3168/jds.2012-5317.

[6] Miyakoshi, Y., Y.Shuji, T.Nagano, Y.Kuroda, Y.Kondo, K.Kondo, K.Tada (1989) Astudy on suckling behavior in piglets. I Effects of birth weight on the teat-order and the amount of milk intake. J.Jpn.Swine Sci.: 26: 203-210.

[7] Neelon,S.E.B., M.Stroo, M.Mayhew, J.Maseiko, C.Hovo (2015) Correlation between mental and infant cortisol varies by brestfeeding status. Infant Behav. Dev.: 40: 252-258. doi.org/10.1016/j. in fbeh.2015.06.005.

[8] Nyachoti, C.M., F.O.Omogbenigun, M.Rademacher, G.Blank (2006) Performance responses and indicators of gastrointestinal health in early-weaned pigs fed-protein amino acid-supplemented diets, J.Anim.Sci.:84:125-134.

[9] Oh, N.S., J.Y.Yeon, J.Y.Lee, J.G.Song, S..Oh, Y.Kim, H.W.Kim, S.H.Kim (2020) Glycated milk protein fermented with lactobacillus rhamnosus ameliorates the cognitive health of mice under mild-stress condition. Gut microbes: 11: 1643-1661. doi.org./10.1080/19490976.2020.1756690.

[10] Rhouma, M., J.M.Fairbrother, F.Beaudry, A.Letellier (2017) Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta.Vet.Scand.: 51: 31-50.

[11] Sotohira, Y., K.Suzuki, T.Sano, C.rai, M.Asakawa, H.Hayashi (2017) Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease. J.Vet.Med.Sci.: 79: 852-854.

[12] Stachowicz,M., A.Lebiedzińska (2016) The effect of diet components on the level of cortisol. Eur.Food res. Technol.: 242: 2001-2009. doi.org/10.1007/s00217-016-2772-3.

[13] Sukemori, S., H.Torii, S.Kisida, M.Tsukada, H.Murakami, T.Oshida (2021) Effect of dietary supplementation of fermented botanical products (FBPs) on coliform bacteria and Salmonella counts in the swine gut, J.Anim.Pro.Enviro.Sci.: 19/20: 56-61.

[14] Tanaka, Y. (2017) Foods with functional claims improving intestinal condition and constipation. Biotechnology :95: 590-593. (in Jpn)

[15] Torii, H., T.Suizu, S.Kishida, M.Tsukada, S.Sukemori (2021) Effects of dietary supplementation of fermented botanical products on the digestibility of crude protein, ether extracts and crude fiber, and carcass characteristics in fattening pigs. Jpn.J.Swine Sci.: 58:142-146.

[16] Uenishi,H., Y.Sato (2013) Physiological mechanism of lactic acid bacteria and its factor. Science of Cookery: 46: 129-133. (in Jpn)

原　著

子豚の離乳ストレスに対する植物発酵製品の影響

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本研究の目的は、ふん便の性状とストレス指標である被毛中のコルチゾール濃度に対する植物発酵製品の給与の影響を評価することである。ふん便の性状は5段階評価の点数を毎日夕方の観察で記録し、被毛中のコルチゾール濃度は生後 21 日目の飼料切り替え初日、新環境に順化したと考えられる育成豚舎移動後 1 週間に当たる生後 50 日目、試験最終日の生後 81 日目に測定した。ふん便の性状には有意な差は認められなかったが、試験区では湿り気の高い、柔らかな正常ふんが多くなる傾向にあり、これは植物発酵製品によって腸管内環境の変化が誘起されたことを示している。本試験成績では対照区の被毛中コルチゾール濃度は下り止まっているが、試験区ではコルチゾールが減少し続けている。有益な発酵産物を含む植物発酵製品は直接あるいは間接的に精神的な健全性にも影響しているかもしれない。

キーワード : 植物発酵製品、腸管内の健康、コルチゾール濃度

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受領日: 14.12.2021.　受理日: 31.01.2022.
日本畜産環境学会会誌 No.21(1) pp14–20. 2022