ผลของสัดส่วนใยอาหารชนิดไม่ละลายน้ำต่อใยอาหารชนิดละลายน้ำในอาหารสุกรหลังหย่านม ต่อน้ำหนักอวัยวะและสัณฐานวิทยาของระบบทางเดินอาหาร

Effects of insoluble per soluble dietary fiber ratio in weaned pig diet on visceral organ weight and gut morphology

นุจิรา ทักษิณานันต์[1], วันดี ทาตระกูล[1*], เสกสม อาตมางกูร[2], รังสรรค์ เจริญสุข[1] และ ทศพร อินเจริญ[1]

Nujira Taksinanan[1], Wandee Tartrakoon[1*], Seksom Attamangkune[2], Rangsun Charoensuk[1] and Tossaporn Incharoen[1]

บทคัดย่อ: ใยอาหารประเภทชนิดไม่ละลายน้ำ (Insoluble dietary fiber; iDF) นั้น มีผลต่อสรีรวิทยาของระบบทางเดินอาหารเป็นอย่างมาก การทดลองครั้งนี้มีวัตถุประสงค์เพื่อศึกษาสัดส่วนใยอาหารชนิดไม่ละลายน้ำต่อชนิดที่ละลายน้ำ (Soluble dietary fiber; sDF) ในอาหาร (iDF:sDF) ต่อการพัฒนาระบบทางเดินอาหารของสุกรระยะอนุบาล โดยใช้สุกรหย่านมจำนวน 64 ตัว อายุ 21± 3 วัน น้ำหนัก 6±0.5 กิโลกรัม แบ่งกลุ่มการทดลองออกเป็น 4 กลุ่มคือ กลุ่มควบคุม (CON) และกลุ่มที่ได้รับอาหารที่มีสัดส่วน iDF:sDF 3 ระดับ คือ 4±0.5 (R4), 5±0.5 (R5) และ 6±0.5 (R6) อาหารทดลองทุกสูตรประกอบสูตรให้ข้าวโพด ปลายข้าว และกากถั่วเหลืองเป็นหลัก บันทึกน้ำหนักของอวัยวะระบบทางเดินอาหาร และตัวอย่างลำไส้เพื่อศึกษาสัณฐานวิทยาในวันที่ 29 หลังจากหย่านม ผลการศึกษาพบว่าการเพิ่มระดับสัดส่วน iDF:sDF จะทำให้น้ำหนักของลำไส้เล็กลดลง (P=0.025) และน้ำหนักของลำไส้ใหญ่เพิ่มขึ้น (P=0.850) ขณะที่น้ำหนักของกระเพาะอาหารไม่มีการเปลี่ยนแปลง (P=0.214) อีกทั้งสุกรที่ได้รับอาหาร R5 และ R6 มีแนวโน้มทำให้ความสูงของวิลไลในลำไส้เล็กส่วนต้นลดลง ส่งผลให้ค่าความสูงวิลไล (Villus height) ต่อความลึกของคริปท์ (Crypt depth) หรือสัดส่วน VH:CD ในลำไส้เล็กส่วนต้นลดลงด้วย (P=0.083) ในส่วนลำไส้เล็กส่วนกลาง การเพิ่มสัดส่วน iDF:sDF มีแนวโน้มทำให้ความสูงของวิลไลสูงขึ้น โดยสุกรที่ได้รับอาหาร R6 มีค่าสัดส่วน VH:CD สูงที่สุด (P=0.004)) โดยสรุปการเพิ่มสัดส่วน iDF:sDF จะส่งผลเชิงลบต่อน้ำหนักของลำไส้เล็กและทำให้ค่าสัดส่วน VH:CD ในลำไส้เล็กส่วนต้นและส่วนกลางลดลง

คำสำคัญ: ใยอาหารที่ไม่ละลายน้ำ, ใยอาหารที่ละลายน้ำ, สุกรหย่านม, สัณฐานวิทยาของลำไส้

---

[1] สาขาวิชาสัตวศาสตร์และเทคโนโลยีอาหารสัตว์ ภาควิชาวิทยาศาสตร์การเกษตร คณะเกษตรศาสตร์ ทรัพยากรธรรมชาติและสิ่งแวดล้อม มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก ประเทศไทย

Animal Science and Feed Technology, Faculty of Agricultural Environmental Science and Natural Resources, Naresuan University, Phitsanulok, 65000, Thailand.

[2] คณะเกษตร กำแพงแสน มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จังหวัดนครปฐม 73140 ประเทศไทย

Faculty of Agriculture, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand.

[*] Corresponding author: wandeeta@nu.ac.th

ABSTRACT: Insoluble dietary fiber (iDF) has many physiological effects on the digestive system. This study aims to investigate effects of the ratio of insoluble per soluble dietary fiber (iDF:sDF ratio) in weaning pig diet on digestive tract development. Total 64 crossbred pigs, weaned at 21±3 days with initial weight about 6±0.5 kg, were randomized to feed four experimental diets: control diet (CON), and 3 differences iDF:sDF ratio: 4±0.5 (R4), 5±0.5 (R5) and 6±0.5 (R6), respectively. All diets were corn-broken rice-soybean meal based diets. The visceral organ weight and gut morphology were collected at 29 days after weaning. The result show increasing iDF:sDF ratio was decreased small intestine weight significantly (P=0.025) and numerically increased large intestine weight (P=0.850) while the weight of stomach was not changed (P=0.214). The duodenal villus height trended to decrease that cause of lower the villus height per crypt depth ratio (VH:CD ratio) in the pig fed R5 and R6 diets (P=0.214). In jejunum, pig fed high iDF:sDF ratio tended to increase villus height. However, the pigs fed R6 diet had greater VH:CD ratio than the others (P=0.004) due to high villus height and less crypt depth. In summary, the increasing of iDF:sDF ratio had negative effect of small intestine weight and decreased the VH:CD ratio in duodenum and jejunum in small intestine.
Keywords: insoluble fiber, soluble fiber, weaned pig, gut morphology

---

Introduction

Weaning is the most critical period for pig by change in intestinal morphology and its functions that usually leads to intestinal mucosal atrophy and harmful bacterial infection. Pig producers might have to use antibiotics in sub-therapeutic level (antibiotics as growth promoter) to control intestinal disease in weaning pig. According the customer concern and antibiotic resistant bacteria situation, using of antibiotic as growth promoter was restricted in animal feed. To improve gut morphology and balance gram negative and gram positive bacteria, one possible method is utilizing dietary fiber via prebiotic effect from feed ingredients. Dietary fiber (DF) is carbohydrate components in plant that are resistant to digestion and absorption in mono-gastric animals and have physical influence the structure of digesta throughout the digestive tract (Perry and Ying, 2016; Williams et al., 2017). Depend on water solubility, dietary fiber is divided into soluble dietary fiber (sDF) and insoluble dietary fiber (iDF). Many researches showed that including iDF in diet had benefits for gut physiology function for example: increase the stomach weight (Gerritsen et al., 2012), increase the weight of large intestine (Hermes et al., 2009), increase mucous secretion and amylase activity and stimulate gut mucosal development (Gerritsen et al., 2012). Increasing iDF:sDF ratio by increase iDF level in diet had some beneficial effects on gastrointestinal tract and its function during 2 weeks after weaning and reduce incidence of post-weaning diarrhea (Pluske et al., 2014). Meanwhile, soluble dietary fiber (sDF) was fermented by microbes in hind gut. The main products are volatile fatty acids that important key to maintain gut pH and control pathogenic bacteria. Many ingredients were studied as insoluble fiber sources in weaning pig diet for example, combination of wheat straw and oat hull (Gerritsen et al., 2012), alfalfa meal (Freire et al., 2000), grass meal (Raj et al., 2005) and sawdust (Lee et al., 2016). In Thailand, rice hull (Oryza sativa L.) is the main by-product from rice processing in rice mills. Total, insoluble and soluble dietary fiber of rice hulls is 74.0%, 72.0% and 2.0% respectively (Jiménez-Moreno et al., 2016).

According of DF composition, rice hull is suitable ingredient as insoluble fiber source in weaning pig diet. This study aims to determine effect of the iDF:sDF ratio on gut morphology and bacterial population in weaning period.

Materials and Methods

The protocols of this study were reviewed and approved by The Center for Animal Research at Naresuan University (NU-AG590507). This study was started during February- March, 2018.

Animals and diets

A total of 64 Duroc x Large White x Landrace weaned pigs, 21 days of age with average initial bodyweight (BW) 6.12 ± 0.12 kg, were provided by local commercial farm. They were randomly divided into 4 groups (16 piglets per group and 4 piglets per pen). All pigs were housed in environmentally controlled rooms with free access to water nipple and feeder. Diet and water were offered ad libitum throughout the duration of the experiment.

Table 1 Ingredients and chemical composition of experimental diets[1/].

Items	CON	R4	R5	R6
Extruded soybean	22.00	22.00	22.00	22.00
Broken rice	31.00	-	-	19.70
Grounded corn	11.60	40.30	11.70	18.20
Soybean meal	18.00	18.00	18.50	18.50
Palm oil	3.00	4.00	4.00	4.00
Milk replacer	5.00	5.00	5.00	5.00
Rice bran	5.00	5.00	5.00	5.00
Rice hull	-	1.00	2.00	3.00
Lysine	0.40	0.40	0.40	0.40
DL-Methionine	0.30	0.30	0.20	0.20
Threonine	0.20	0.20	0.20	0.20
DCP	2.00	2.00	2.00	2.00
NaCl	0.50	0.50	0.50	0.50
$CaCO_3$	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2/]	0.50	0.50	0.50	0.50
Chemical composition (as fed basis)				
DM, %	89.74	90.25	90.22	89.80
CF, %	2.80	4.05	4.49	4.73
Total dietary fiber, %	10.88	13.69	12.87	12.54
Insoluble dietary fiber, %	9.17	10.98	10.72	10.75
Soluble dietary fiber, %	3.05	2.75	2.14	1.79
iDF:sDF ratio	3.00	4.00	5.00	6.00

[1/] CON = control diet, R4 =diet with iDF:SDF ratio =4, R5= diet with iDF:sDF ratio=5 and R6= diet with iDF:sDF ratio =6.
[2/] each 1 kg contain Vitamin A 10 MIU, Vitamin D3 2 MIU, Vitamin E 20,000 IU, Vitamin K3 1.60 g, Vitamin B1 1.20 g, Vitamin B6 2.00 g, Vitamin B12 0.016 g, Pantothenic acid 10.00 g, Niacin 16.00, Folic acid 0.40 g, Biotin 0.06 g, Vitamin C 0.06 g, Selenium 0.10 g, Iron 72.00 g, Manganese 20.00 g, Zinc 60.00 g, Copper 64.00 g, Cobalt 0.32 g, Iodine 0.06 g, Preservative 2.50g, Additive 10.00 g.

As shown in Table 1, all diets were formulated to meet the nutrient requirement of pigs (NRC, 2012). Diets were corn, broken rice and soybean-based diet and fed as mash form. Pigs were fed the following diets: 1) control diet without rice hull (CON), 2) diet with iDF;sDF ratio = 4.00 $\pm$ 0.5 by adding 1% rice hull (R4), 3) diet with iDF;sDF ratio = 5.00 $\pm$0.5 by adding 2% rice hull (R5), 4) diet with iDF:sDF ratio = 6.00 $\pm$ 0.5 by adding 3% rice hull (R6). Rice hull (Oryza sativa L.), as represent insoluble fiber source, brought from local dealer. Then, washed, dried and ground with hammer mill (Retsch model SM 100) through sieve opening size 0.5 mm before mixing. L-Lysine, DL-Methionine and L-Threonine were provided in quantities meeting the animal requirement. No antibiotic growth promoter (AGP) added in the diets.

Sample collection and analysis

Diet samples were ground through a 0.5-mm mesh screen for chemical analysis. The dry matter and crude fiber were determined according AOAC (1995). Total dietary fiber (DF) and insoluble dietary fiber (iDF) were determined according AOAC 991.45. Soluble dietary fiber (sDF) was estimated as the different between DF and iDF (Montagne et al., 2012)

On day 29, a pig per pen (n=4) was randomized, weighed and euthanized. The abdomen was opened immediately and gastrointestinal tract was removed. Empty weight of the stomach, small and large intestine and liver were recorded. The section of duodenum (approximately 10 cm from stomach sphincter), jejunum (5.5 m from stomach sphincter) and ileum (10 cm prior to the ileo-caecal junction) were sampling and fixed suddenly in 10% formalin buffer. The intestinal samples were dehydrated, embedded in paraffin. Transverse sections were cut at 5 μm thickness and stained with haematoxylin and eosin. Twelve complete villi and crypt were selected and measured at 40X magnification. Villi height was the distance from the top of the villus to the villus-crypt junction and crypt depth was considered as the invagination depth between adjacent villus.

All data were analyzed as randomized design using SPSS statistical software (Ver. 15 for Windows, SPSS Inc., Chicago, IL, USA). The difference between means was assessed by ANOVA and Duncan's Multiple Range Test was then used to compare data among treatments. The statistical significance between treatments was based on $P < 0.05$. Tendencies $0.05 < P < 0.10$ were also presented.

Results and Discussion

Gut development and morphology

The weight stomach and large intestine were not affected by experimental diets (Table 2). Increasing iDF:sDF ratio was significantly decreased small intestine weight (P= 0.025). This result was probably due to faster transit of digesta along gastro-intestinal tract when increase iDF ratio. Suggestion by Hambrecht (1998), increasing slow and poor fermentable fiber source could be induced low production rate of volatile fatty acids. Thus, it can be explained the lower weight of small intestine and not significant of large intestine weight. Similar to previous research, fiber intake had negative correlation with length and weight of small intestine in pig (-0.68 and -0.45) but had positive correlation with length and weight of large intestine (0.20 and 0.23) (Raj et al., 2005).

As shown in Table 3, whereas the crypt depth wasn't differing among treatments, the duodenal villus height tended to decrease when increasing the iDF:sDF ratio (P = 0.083). Therefore, the VH:CD ratio was significantly differing among treatments. The VH:CD ratio of pigs fed R4 diet was increased and decreased in R5 and R6 group. In jejunum, pigs fed higher iDF:sDF ratio tended to increase villus height (P = 0.092) but significantly decrease crypt depth and VH:CD ratio (P = 0.004). The experimental diets did not affect morphology of ileum.

Table 2 Weight of stomach, small and large intestine (g) of pigs fed difference iDF:sDF ratio after weaning.

items	Control	R4	R5	R6	SEM	p-value
Body weight, kg	15.2	14.8	14.6	15.0	0.200	0.671
Organ weight, g						
Stomach	186.67	183.33	163.33	183.33	3.38	0.214
Small intestine	1,063.3^{b}	990.0^{ab}	880.0^{a}	895.0^{a}	16.99	0.025
Large intestine	545.0	516.67	526.67	526.67	10.90	0.850

^{a,b} Within a row, means without a common superscript differ significantly (P < 0.05).

After weaning, epithelial cell of digestive system is destroyed by many factors and its take five to eight days after weaning, villus height began to increase (Pluske et al., 1997). This stage was direct affect nutrient digestion, absorption and gut morphology. Moreover, epithelial cells of digestive system play important role to be the barrier protecting the colonization of harmful bacteria. From the result of this study, experimental diets had an impact on small intestinal morphology especially duodenum and jejunum. This could be due to increase iDF content in diet caused high passage rate and less substrate for microbial fermentation. Thus, the villi height and VH:CD ratio was lower in duodenum.

Table 3 Villus height, Crypt depth and VH:CD ratio of pig fed different iDF:sDF ratio during nursery period

items	Control	R4	R5	R6	SEM	p-value
Duodenum						
Villous height, μ	326.89	293.99	255.13	256.26	10.21	0.083
Crypt depth, μ	146.52	114.17	134.34	142.46	6.09	0.292
VH:CD	2.36^{bc}	2.61^{c}	2.07^{ab}	1.81^{a}	0.08	0.016
Jejunum						
Villous height, μ	359.13	280.40	285.98	317.56	10.99	0.092
Crypt depth, μ	141.78^{bc}	127.50^{ab}	149.41^{c}	110.57^{a}	3.13	0.004
VH:CD	2.48^{b}	2.31^{ab}	1.92^{a}	3.02^{c}	0.08	0.004
Ileum						
Villous height, μ	218.57	225.29	205.98	235.06	10.95	0.817
Crypt depth, μ	131.90	119.59	132.65	119.47	5.09	0.675
VH:CD	2.23	2.06	1.77	1.87	0.07	0.196

VH:CD = the ratio of villus height to crypt depth.

^{a,b,c} Within a row, means without a common superscript differ significantly (P < 0.05).

Conclusion

Increasing the iDF:sDF ratio in weaning pig diet decrease the small intestine weight and influence on gut morphology especially duodenum and jejunum.

Acknowledgements

This research is sponsored and supported by National Research Council of Thailand. The authors would like to thank the researchers and staffs at Faculty of Agricultural Environmental Science and Natural Resources, Naresuan University and Laboratory of Animal Nutrition of Department of Animal Science, Rajamangala University of Technology Lanna for their help in carrying out the study.

References

AOAC. 1995. Official Methods of Analysis, Association of Official Analytical Chemists. 15th Edition. Arlington, VA.

Freire, J. P. B., A. J. G. Guerreiro, L. F. Cunha, and A. Aumaitre. 2000. Effect of dietary fibre sources on total tract digestibility, caecum volatile fatty acids and digestive transit time in the weaned piglet. Anim. Feed Sci. Tech. 87:71-83.

Gerritsen, R., P. Van der Var, and F. Molist. 2012. Insoluble non-starch polysaccharides in diets for weaning piglets. J. Anim. Sci. 90:318-320.

Hambrecht, E. 1998. Effect of non-starch polysaccharides on performance, incidence of diarrhea and gut growth in weaned pigs. M. S. Thesis. University of Hohenheim, Stuttgart.

Hermes, R. G., F. Molist, M. Ywazaki, M. Nofrarias, G. S. Gomez, J. Gasa, and J. F. Perez. Effect of dietary level of protein and fiber on the productive performance and health status of piglet. J. Anim. Sci. 87:3569-3577.

Jiménez-Moreno, E., A. de Coca-Sinova, J. M. González-Alvarado, and G. G. Mateos. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers 1. Effects on growth performance and water intake. Poult. Sci. 95:41-52.

Lee, S. J., J. Y. Kim, N. H. Park, E. G. Awji, J. W. Suh and S. C. Park. 2016. The effect of dietary fiber source on the growth performance and intestinal microflora in piglets. Intern. J. Appl. Res. Vet. Med. 14:135-142.

Montagne, L., N. Le Floc'h, M. Srturo-Schaan, R. Foret, M. C. Urdaci, and M. Le Gall. 2012. Comparative effects of level of dietary fiber and sanitary conditions on the growth and health of weanling pigs. J. Anim. Sci. 90:2556-2569.

NRC. 2012. Nutritional Requirements of Swine. 11th edition. National Academy Press, Washington DC.

Perry, J. R. and W. Ying. 2016. A review of physiological effects of soluble and insoluble dietary fibers. J. Nutr. Food Sci. 6:476.

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: A review. Livest. Prod. Sci. 51:215-236.

Pluske, J., J. C. Kim, R. Hewitt, and R. Barneveld. 2014. Reducing the risk of post-weaning E. coli diarrhea using different sources of fibre in diets. http://porkcrc.com.au/wp-content/uploads/2014/12/2A-105-Final-Report.pdf. Accessed 12 Apr. 2018.

Raj, St., G. Skiba, D. Weremko, and H. Fandrejewski. 2005. Growth of the gastrointestinal tract of pigs during realimentation following a high fibre diet. J. Anim. Feed Sci. 14:675-684.

Williams, B. A., I. J. Grant, M. J. Gidley, and D. Mikkelsen. 2017. Gut fermentation of dietary fibres: Physico-chemistry of plant cell walls and implication for health. Int. J. Mol. Sci. 18:2203.