Université de Liège

Gembloux Agro-Bio Tech

The effects of xylo-oligosaccharides on gut barrier function, intestinal microbiota and growth Performance in weaned piglets

Chen Yuxia

COMMUNAUTÉ FRANÇAISE DE BELGIQUE

UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

The effects of xylo-oligosaccharides on gut barrier function, intestinal microbiota and growth performance in weaned piglets

Yuxia Chen

Dissertation originale présentée en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur(s): Prof. Yves BECKERS,
Co-promoteur : Prof. Nadia EVERAERT (KU Leuven),
Co-promoteur : Prof. Hongfu ZHANG,
Année civile: 2022

Abstract

Weaning piglets in modern swine industry is often challenged by post-weaning stresses including dietary, social, and environmental changes. These stresses result in increasing disease and mortality risks such as post-weaning diarrhea. This also raises tremendous physiological, immunological and microbiological changes in the piglet's intestines. To promote animal growth and prevent infections, antibiotics are widely used in weaned piglets. Unfortunately, the usage of antibiotics as feed additives for long periods in animal diets can lead to antibiotic resistance problem and high residue levels in animal products. Since the quest for safer and healthier meat was remarkably increased in recent time, the use of nutritional feed additives is considered as promising strategy to alleviate intestinal disturbances around weaning. Prebiotics from the agricultural by-products are nutrients that have the potential to considerably influence the physiology of the whole body and, consequently, health, and well-being. Xylo-oligosaccharides (XOS) are considered as functional oligosaccharides and have great prebiotic potential. However, the optimal XOS supplementation dosage used in the diet of weaned piglets is still unknown. Therefore, the aims of the research described in this thesis are to (1) select an optimal XOS supplementation dosage that improve the growth performance, serum parameters, small intestinal morphology, intestinal mucosal integrity, and immune function in weaned piglets. (2) investigate whether optimal XOS supplementation dosage as potential replacements for antibiotic optimizes the gut morphology, gut microbial and metabolic composition of weaned piglets.

For the first objective, a total of 240 weaned piglets with an average body weight (BW) of 8.82 ± 0.05 kg (28 d of age) were assigned randomly to 4 dietary treatments in a 28-d trial, including a control diet (CON), 3 diets with XOS supplementation at the concentration of 100, 500 and 1000 mg/kg (XOS100, XOS500, and XOS1000). There were 4 replicates per treatment with 15 pigs per pen. The different XOS dose groups showed a quadratic effect on BW on day 28, ADG, and G:F day 1 to -28 of piglets ($P < 0.05$). From d 15 to 28, ADG of pigs fed the XOS500 diet was higher ($P < 0.05$) than pigs fed the CON diet. During the overall period (d 1 to 28), pigs fed the XOS500 diet had a higher BW, ADG and G:F than pigs fed the CON diet ($P < 0.05$). In addition, compared with the CON group, the XOS500 group had significantly higher serum antioxidant capacity on d 14 and 28. Supplementation of XOS500 to the feed significantly improved intestinal morphology in the jejunum and ileum in comparison with the CON and XOS1000 group. Moreover, the XOS500 group significantly elevated the expression levels of Occludin and zonula occludens protein-1 (ZO-1) in the ileum compared to the CON group. The ileal proinflammatory cytokine expression levels in the XOS100 and XOS500 group were markedly lower than in the CON group. In contrast, the ileal IL-10 mRNA expression levels were remarkably higher in the XOS500 than CON group. The results indicated that the optimal level of xylo-oligosaccharides have a beneficial effect on growth performance by improving serum antioxidant defense system, serum IgG, small intestinal structure and intestinal barrier function in weaned piglets.

Secondly, we investigate whether optimal XOS supplementation dosage as potential replacements for chlortetracycline optimizes the gut morphology, gut microbial and metabolic composition of weaned piglets. A total of 180 weaned piglets were randomly allocated to three treatments for 28 days, as follows: control group (basal diet, CON), basal diet with 500mg/kg

(XOS500) XOS, and positive control (basal diet with 100 mg/kg chlortetracycline, CTC). Compared with the CON group, the piglets in the XOS500 group improved growth performance during days 1–28 ($P < 0.05$). High-throughput 16S rRNA gene sequencing revealed distinct differences in microbial compositions between the ileum and cecum. XOS500 supplementation significantly increased the bacterial diversity. However, CTC treatment markedly reduced the microbial diversity ($P < 0.05$). Meanwhile, XOS500 supplementation in the diet significantly increased the abundance of *Lactobacillus* genus compared to the CON and CTC group in the ileum and cecum ($P < 0.01$), whereas the level of *Clostridium_sensu_stricto_1*, *Escherichia-Shigella* and *Terrisporobacter* genus in the XOS500 group were markedly lower than the CON and CTC group ($P < 0.05$). In addition, dietary supplementation with XOS500 significantly increased the total short-chain fatty acids, propionate and butyrate concentrations and decreased the acetate concentration compared to the CON group in the cecum ($P < 0.05$). The results indicated that dietary supplemented with XOS500 could enhance specific beneficial microbiota abundance and decrease harmful microbiota abundance to maintain the structure of the intestinal morphology and improve growth performance of weaned piglets. Thus, XOS may potentially function as an alternative to in-feed antibiotics in weaned piglets in modern husbandry.

In summary, this thesis demonstrated that XOS supplementation could function in a dose-dependent manner. The optimal XOS supplementation dosage could improve serum antioxidant capacity, immune function, maintain intestinal barrier function, and enhance specific beneficial microbiota abundance and decrease harmful microbiota abundance, changes SCFA production which resulted in maintain the structure of the intestinal morphology and improve growth performance of weaned piglets. Thus, XOS may potentially function as an alternative to in-feed antibiotics in weaned piglets in modern husbandry. This work may not only provide insight into strategies for improving the status of weaned piglets targeting the gut microbiota, but also help us to develop a complementary understanding of effective feed additives for weaned piglet health.

Keywords: Xylo-oligosaccharides; Growth performance; Gut microbiota; Antibiotics; Weaned piglets

Résumé

Les porcelets sevrés dans l'industrie porcine moderne sont confrontés à des stress post-sevrage, liés notamment aux changements alimentaires, sociaux et environnementaux. Ces stress entraînent une augmentation des risques de maladie et de mortalité tels que la diarrhée post-sevrage. Cela entraîne également d'énormes changements physiologiques, immunologiques et microbiologiques dans les intestins du porcelet. Pour favoriser la croissance des animaux et prévenir les infections, les antibiotiques sont largement utilisés chez les porcelets sevrés. Malheureusement, l'utilisation d'antibiotiques comme additifs alimentaires pendant de longues périodes dans les régimes alimentaires des animaux peut entraîner des problèmes de résistance aux antibiotiques et des niveaux élevés de résidus dans les produits d'origine animale. Étant donné que la quête d'une viande plus sûre et plus saine s'est considérablement accrue ces derniers temps, l'utilisation d'additifs alimentaires nutritionnels est considérée comme une stratégie prometteuse pour atténuer les troubles intestinaux autour du sevrage. Les prébiotiques issus des coproduits agricoles sont des nutriments qui ont le potentiel d'influencer considérablement la physiologie de tout le corps et, par conséquent, la santé et le bien-être. Les xylo-oligosaccharides (XOS) sont considérés comme des oligosaccharides fonctionnels et ont un grand potentiel prébiotique. Cependant, la dose optimale de supplémentation en XOS utilisée dans l'alimentation des porcelets sevrés est encore inconnue. Par conséquent, les objectifs de cette recherche doctorale sont (1) de sélectionner une dose optimale de supplémentation en XOS qui améliore les performances de croissance, les paramètres sériques, la morphologie de l'intestin grêle, l'intégrité de la muqueuse intestinale et la fonction immunitaire chez les porcelets sevrés. (2) d'étudier si la dose optimale de supplémentation en XOS en tant que substituts potentiels de l'antibiotique optimise la morphologie intestinale, la composition microbienne et métabolique de l'intestin des porcelets sevrés.

Pour le premier objectif, un total de 240 porcelets sevrés avec un poids corporel moyen (PC) de 8,82 ± 0,05 kg (28 jours d'âge) ont été assignés au hasard à 4 traitements alimentaires dans un essai de 28 jours, comprenant un régime contrôle (CON), 3 régimes avec supplémentation en XOS à la concentration de 100, 500 et 1000 mg/kg, respectivement (XOS100, XOS500 et XOS1000). Il y avait 4 répétitions par traitement et 15 porcs par loge. Les régimes supplémentés en XOS ont montré un effet quadratique sur le PC au jour 28, la croissance quotidienne (ADG) et le rapport gain de poids : aliments consommés (G:F) du jour 1 au jour 28 des porcelets ($P < 0,05$). Du jour 15 au jour 28, l'ADG des porcs nourris avec le régime XOS500 était plus élevé ($P < 0,05$) que celui des porcs nourris avec le régime CON. Au cours de la période globale (j 1 à j 28), les porcs nourris avec le régime XOS500 avaient un PC, un ADG et un G:F plus élevés que les porcs nourris avec le régime CON ($P < 0,05$). De plus, par rapport au groupe CON, le groupe XOS500 avait une capacité antioxydante sérique significativement plus élevée aux jours 14 et 28. La supplémentation en XOS500 de l'aliment a significativement amélioré la morphologie intestinale dans le jéjunum et l'iléon par rapport au groupe CON et XOS1000. De plus, le groupe XOS500 a significativement élevé les niveaux d'expression de l'Occludine et de la protéine zonula occludens-1 (ZO-1) dans l'iléon par rapport au groupe CON. Les niveaux d'expression des cytokines pro-inflammatoires iléales dans les groupes XOS100 et XOS500

étaient nettement inférieurs à ceux du groupe CON. En revanche, les niveaux d'expression de l'ARNm de l'IL-10 iléale étaient remarquablement plus élevés dans le groupe XOS500 que dans le groupe CON. Les résultats démontrent que le niveau optimal des xylo-oligosaccharides a un effet bénéfique sur les performances de croissance en améliorant le système de défense antioxydant du sérum, les IgG sériques, la structure de l'intestin grêle et la fonction de barrière intestinale chez les porcelets sevrés.

Durant la seconde expérience, la dose optimale de XOS est comparée aux effets de la chlortétracycline sur la morphologie intestinale, la composition microbienne et métabolique de l'intestin des porcelets sevrés. Un total de 180 porcelets sevrés ont été répartis au hasard entre trois traitements pendant 28 jours: groupe témoin (régime de base, CON), régime de base avec 500 mg/kg de XOS (XOS500) et le régime de base avec 100 mg/kg chlortétracycline (témoin positif, CTC). Par rapport au groupe CON, les porcelets du groupe XOS500 ont amélioré leurs performances de croissance pendant les jours 1 à 28 ($P < 0,05$). Le séquençage à haut débit du gène de l'ARNr 16S a révélé des différences distinctes dans les compositions microbiennes entre l'iléon et le caecum. La supplémentation en XOS500 a significativement augmenté la diversité bactérienne alors que le traitement CTC l'a nettement réduite ($P < 0,05$). La supplémentation en XOS500 dans l'alimentation a augmenté de manière significative l'abondance du genre Lactobacillus par rapport aux groupes CON et CTC dans l'iléon et le caecum ($P < 0,01$), alors que le niveau de Clostridium_sensu_stricto_1, Escherichia-Shigella et Terrisporobacter dans le groupe XOS500 était nettement inférieur aux groupes CON et CTC ($P < 0,05$). De plus, la supplémentation alimentaire avec XOS500 a augmenté de manière significative les concentrations totales d'acides gras à chaîne courte, de propionate et de butyrate et a diminué la concentration d'acétate par rapport au groupe CON dans le caecum ($P < 0,05$). Les résultats ont indiqué qu'un régime alimentaire supplémenté avec XOS500 pourrait améliorer l'abondance spécifique du microbiote bénéfique et diminuer l'abondance du microbiote nocif pour maintenir la structure de la morphologie intestinale et améliorer les performances de croissance des porcelets sevrés. Ainsi, les XOS peuvent potentiellement fonctionner comme une alternative aux antibiotiques dans l'alimentation des porcelets sevrés dans l'élevage moderne.

En résumé, cette thèse a démontré que la supplémentation en XOS pouvait fonctionner d'une manière dose-dépendante. Le dosage optimal de la supplémentation en XOS pourrait améliorer la capacité antioxydante du sérum, la fonction immunitaire, maintenir la fonction de barrière intestinale et améliorer l'abondance du microbiote bénéfique spécifique et diminuer l'abondance du microbiote nocif, modifier la production de SCFA, ce qui a permis de maintenir la structure de la morphologie intestinale et d'améliorer les performances de croissance des sevrés. porcelets. Ainsi, les XOS peuvent potentiellement fonctionner comme une alternative aux antibiotiques dans l'alimentation des porcelets sevrés dans les élevages modernes. Ce travail peut non seulement fournir un aperçu des stratégies d'amélioration du statut des porcelets sevrés ciblant le microbiote intestinal, mais aussi nous aider à développer une compréhension complémentaire des additifs alimentaires efficaces pour la santé des porcelets sevrés.

Mots-clés: Xylo-oligosaccharides; Performances de croissance; Microbiote intestinal; Antibiotiques; Porcelets sevrés

Acknowledgements

First of all, I would like to express my deepest gratitude to my supervisor and co-supervisors: Prof. Yves Beckers, Prof. Nadia Everaert and Prof. Hongfu Zhang (CAAS). Thanks for your continuous support of my Ph.D study and related researches, and for your patience and motivation. Your guidance helped me in realization of this work successfully.

I would also like to express my gratitude to my thesis committee and jury members including president Prof. Jerome Bindelle, members: Yves Beckers, Nadia Everaert, Hongfu Zhang, Martine Laitat, Martine Schroyen. Thanks for taking your valuable time to read, evaluate and give pertinent suggestions to my research and thesis. Thanks for all your valuable time and genuine help!

Since September 1st, 2017, I have officially become a doctoral candidate jointly cultivated by the Chinese Academy of Agricultural Sciences and the University of Liege. It has been about 4 years and 9 months so far. Time is flying! I started my PhD studying with a good yearning for the future. I also understand that PhD learning is a difficult challenge for me. Looking back on the past, I found that all of this is traceable.

I remembered that the first time Prof. Yves sent me email at the end of May 2018 and reminded me to submit the annual Activity Report. The second time I had forgotten my student login password for the University of Liège. Under your help, I got my student login password. I remembered that Yves supervised me to revise my private defense powerpoint and my manuscript with patience. Thank you!

I remembered that the first time Prof. Nadia sent an email to remind me to submit an Activity Report in May 2019. At the beginning of the epidemic in 2020, I encountered registration problems again. After hard communication, I am finally qualified to continue my PhD study. I cherish this opportunity for my PhD study. I remembered that you supervised me to revise my English articles with patience. In the meanwhile, all these past events reminded me that I must express my sincere thanks all of you. Thank you!

I am very grateful to Prof. Zhang for giving me this opportunity to study for a joint Ph.D between the Chinese Academy of Agricultural Sciences and the University of Liege. I remembered that the day I met you at the door of your office and it was also the first day you allowed me into the laboratory. A professor from Purdue University, Adeola, who came to the laboratory to cooperate and exchange, that time I was impressed. For the first time, I felt the excitement of cooperating and communicating with international friends. I remembered when I raised pigs first time, you recommended me to contact the farm and gave me patient guidance when I was in trouble. You have given me a lot of financial help and spiritual encouragement in my life. During these years in the lab, Hongfu not only taught me how to do scientific research, but also how to be a more positive person, a more valuable person. Hongfu always encourages us to be proactive and courageous to express ourselves, to be diligent and kind. I will practice your wonderful teachings in my whole life! Thank you!

I would also like to thank Prof. Liang Chen for the help of experimental design and thesis writing. I learned many important aspects of conducting a research project successfully to its end, and thank him for the long and instructive discussions that we had over the years about and around my PhD project. Thank you for your care and support in the life and study during my doctoral studies!

I would further express many thanks to Yining Xie, Prof. Kolapo M. Ajuwon, Prof. Yong Zhao, Dr. Ruqing Zhong, Lei Liu, Dr. Shanlong Tang, Lilan Zhang, Dan Shen, Huaibao Zhao, Xiaobin Wen, Shunfen Zhang, Hui Han, Fan Wan, Kai Li, Bifeng Zhang.

I am also very grateful to the staff of the State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (IAS, CAAS, Beijing) for all the help given. Thank all the members from Precision Livestock and Nutrition Unit of Gembloux Agro-Bio tech. Thank you all for welcoming me, for your support, for your kindness and your friendship. Additionally, I wish to thank China Scholarship Council (CSC) for the financial support to study in Belgium.

I would like to thank my uncle Director of the Livestock Bureau Fengjun Liu for bringing me into the major of animal nutrition and feed science, and for your care and help throughout my learning process!

I would like to thank Dr. Yulong Feng for your guidance and help during my Ph.D. exams!

I would like to thank Prof. Kolapo M. Ajuwon for your guidance and help for my English review and other articles. Thanks to your encouragement and warmth during my doctoral life!

I would like to thank General Manager Tao Li who provided support and help during my doctoral project!

I would like to thanks to my friend Dr. Anran Wang for being with us all the way. We prepared materials for going Belgium together, stepped into this unfamiliar land of Belgium together, lived and studied here together, and experienced the local customs together. We have experienced too many first times together. We encouraged each other, helped each other, growed together, witnessed each other, and we all became better versions of ourselves. Thank you for being with you on this journey!

I would like to thank my Belgian hosts, Mr. and Mrs. Hamende Jean Pierre, for your care and help! I will cherish this friendship that spans ages and borders, as well as the wonderful memories this humorous, friendly and kind your left me!

I would like to thank General Manager Changguang Lin, General Manager Jinyu Lin, Prof. Yijian Wu, and Director Mengjie Zhang who provided support and help in the animal experiments.

I would also like to thank Director Mingjun Zhang who are responsible for between the CAAS and Liege University project. Thank you for your support and help!

I would also like to thank Dean Lin Xiao and Xinming Wang Longlive Biotechnology Company for providing some materials and solving some doubts in the defense. Thank you!

I would like to thank President Ming Sun of Beijing agriculture and animal husbandry enterprise entrepreneurship and Development Alliance for his help and support during period of studying abroad!

I would like to thank my master Guowei Yuan, a famous pig raising expert in China at present, for your supervising and answering to questions on the front line of pig raising. Thank you!

I would like to thank President Weidong Jin and Jiahui Qiu of Wellhope Food Co., Ltd. for his help and support during period of studying abroad!

I would also like to thank Shuang Ye, a young Chinese diplomat in South Sudan now, for your help, guidance and warmth during my studying in Belgium. You shared your alarm clock "Brian Wetzel/Andrew Wysong" helped me dispel my fears. Your encouragement and inspiration gave me much courage to face and overcome my fears. Thank you for your careful

guidance on my essay about the cross-border friendship, which ended up winning second prize for “The 50th Anniversary of the Establishment of Diplomatic Relations between China and Belgium”.

I would like to thank my friend Lichuan Gao, one Senior Manager of New China Insurance, for your guidance in reciting the video sound, and finally won the second prize in the online recitation competition for “Chinese students studying in China for the 100th anniversary of the founding of the Communist Party of China”.

I would like to thank my friend Lintao Xu, one General Manager of China Mobile, for your giving me so much positive mental energy. Thank you!

I would like to thank Professor Eloy for your giving me many suggestions and revising my English article. Thank you!

I would like to thank my friend Dr. Junnan Song for your generous sharing and encouragement during my Belgium studying. You are a positive, excellent and very good girl. Thank you!

My most affectionate thanks go to my family. I would like to thank my husband Dr. Liyuan Wang and you give me so much love and support! It was my luck to meet you at my most beautiful age when I was a graduate student and to hold hands with you. I learned from you so much. We got married during our Ph.D. studying, you are always the first person I think of when I encounter difficulties. Of course, when I got achievement, the first person I want to share is you. Thank you for your patience, kindness and gentleness! You gave me the courage and the energy to go through my doctoral career! I am very fortunate to be lovers, couples, friends and comrades in work and life with you! I love you, thank you!

Finally, I would like to thank sincerely all the people who helped me to carry it out successfully and to be a better person in my life!

Stay hungry, stay foolish.

-- Steve Jobs

Tables of Contents

List of Figures

List of Tables

List of Abbreviations

XOS	Xylo-oligosaccharides
BW	body weight
ADG	average daily gain
ADFI	average daily feed intake
G:F	gain to feed ratio
T-AOC	total antioxidant capacity
T-SOD	total superoxide dismutase
GSH-Px	glutathione peroxidase
MDA	malondialdehyde
CAT	catalase
IgG	Immunoglobulin G
IgA	immunoglobulin A
IgM	immunoglobulin M
VH	villus height
CD	crypt depth
VH:CD	villus height to crypt depth
ZO-1	zonula occluden protein-1
IL-1β	interleukin-1β
IFN-γ	interferon-γ
AGP	antibiotic growth promoters
FCR	feed conversion ratio
qRT-PCR	quantitative real-time polymerase chain reaction
SCFA	short chain fatty acid
GH	glycoside hydrolase

1

General introduction

General introduction

1. Introduction

1.1. Pig production

The global pork production is of great significance in the agricultural market. The huge consumer demand for pork has greatly stimulated the pork production sector, especially in recent decades. The proportion of pork in the consumption of various meats is very high in all countries. At present, pork is ranked as second after poultry meat (Popescu, 2020). China's fast increasing economy has provided people with higher purchasing power, resulting in a rapid expansion of the Chinese swine industry over the past decades. China is also the world's largest pork producer. In 2017, pork production in China is up to 54.5 million metric tons which is estimated to nearly 50% of the total pork production in the world. Because the rapid spread of African Swine Fever (ASF) across China shrunk the world's largest swine production in 2019. Overall Chinese swine production dropped to 36.3 million metric tons which accounts for only 38% of the world's proportion in 2020 as ASF continue (Figure 1-1 and Figure 1-2). The main regions of pig production are the southwestern and northern areas of China and south of the Huaihe River (Wu, 2018). The top five provinces including Sichuan, Henan, Hunan, Shandong, and Yunnan which account for > 40% of the total pig production in 2020 (Figure 1-2 and Figure1-3).

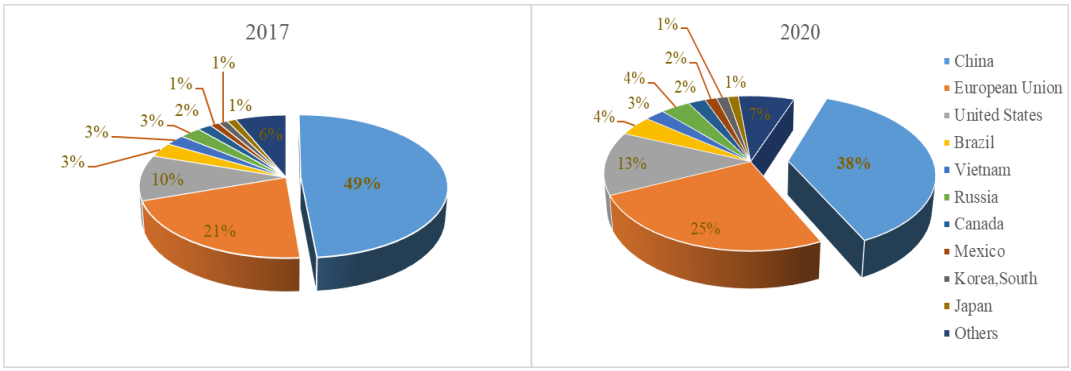


Figure 1-1. Top ten pork producing countries in 2017 and 2020. Source: USDA-FAS-PSD.

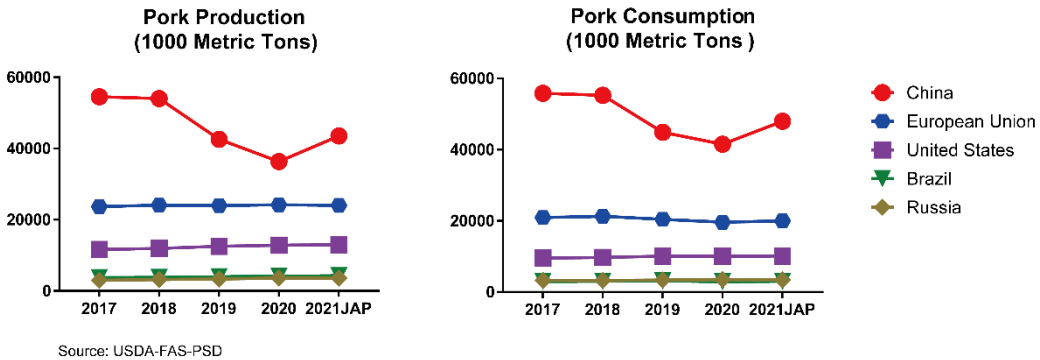
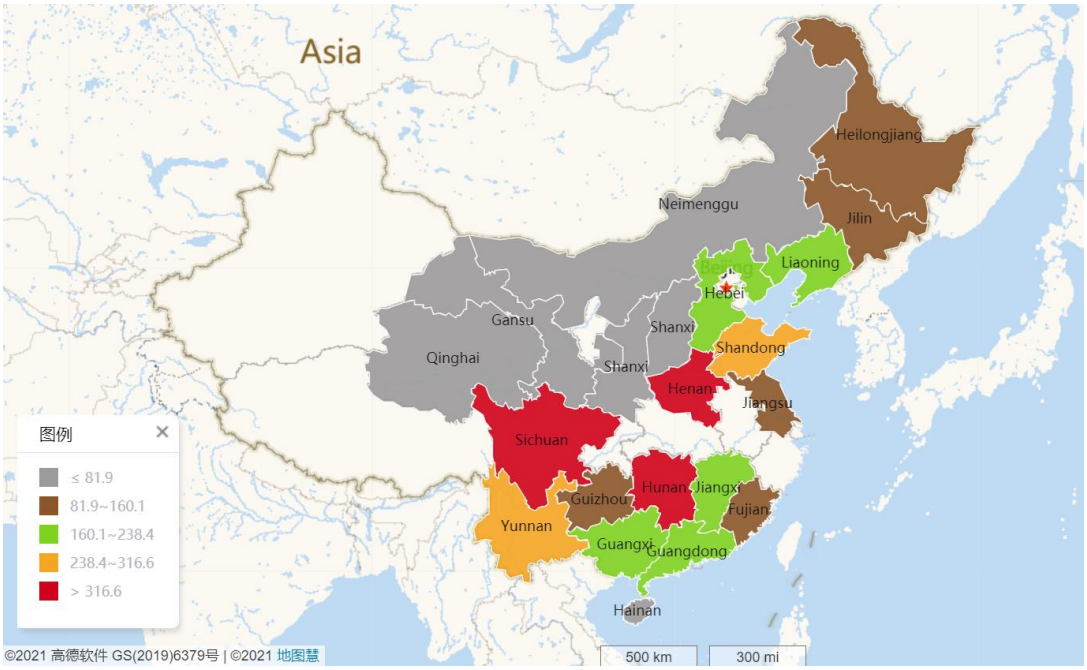


Figure 1-2. Change in world pork production from 2017-2021 (1000 metric tons).



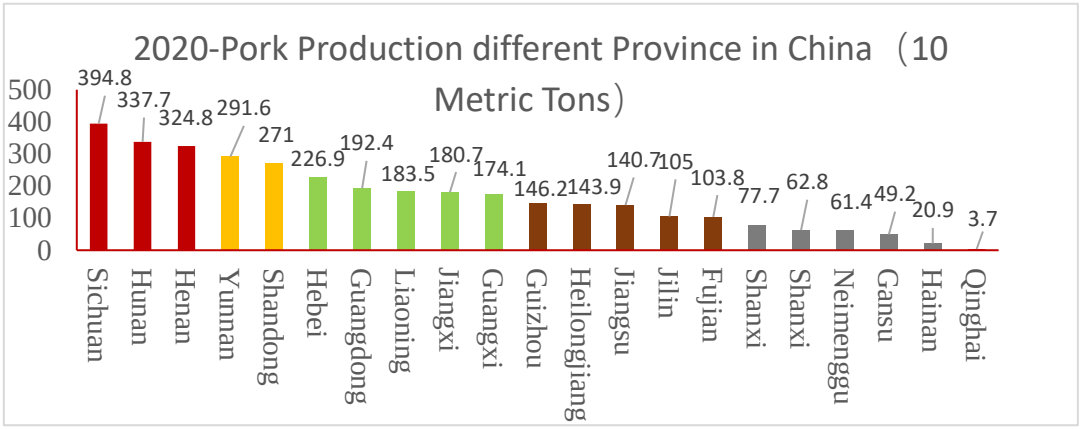


Figure 1-3. Regional distribution of pork production in China in 2020 (10 metric tons). Source: National Bureau of Statistics of China.

Why does China have just over 18% of the world's population, but its pork consumption has reached nearly 50% of the total pork produced in the world? According to historical records, around 6,000 BC, pig farming has become popular in the Central Plains area in China (Zhang, 1993). The domestic pig breeding industry developed rapidly in the Western Han Dynasty. In addition, the importance of pigs to the Chinese people can be further explained by the Chinese character "家=house" in the Oracle bone inscriptions. From the perspective of the font, the upper part of it "宀" represents the house, and the lower part of it "豕" represents the pig. In ancient times, people only raising pigs under the house could be called a home. This shows the importance of pigs to a family.

1.2. The rearing practices of pigs

Doing a good job in raising piglets in a pig farm can effectively improve the ability of piglets to resist stress, enhance the resistance of piglets, and ensure the health of piglets, thereby improving the economic benefits of the farm. Piglets are easy to catch a cold once they leave the sow. Therefore, the temperature of the piglets should be kept in time after weaning. The optimum temperature of weaned piglets is 21-22°C in 30-40d, the temperature is controlled at

21°C in 41-60d, and the temperature is not lower than 20°C in 60-90d. Therefore, in order to achieve the above piglet temperature in the weaned piglet house, warm measures should be taken in winter. In addition to paying attention to the windproof and heat insulation of the house and increasing the number of feedings in the house, it is best to install heating equipment such as air conditioners, hot blast stoves, and coal-fired stoves. On the contrary, it is necessary to lower the temperature in hot summer. Pig farms can adopt methods such as pig house ventilation and spraying. High humidity in piglet houses can adversely affect piglets. This is because high humidity is conducive to the reproduction of pathogenic microorganisms and may lead to the occurrence of various diseases in piglets. In general, the optimum humidity for weaned piglets is 65% to 75% (Sun 2021; Wang 2021).

Three to four weeks postpartum sow is the peak lactation period, and more than 65% of the sow's milk is consumed by piglets of four to five weeks old. Generally speaking, 84% of the nutritional needs of piglets can be met 30 days after sows, only 50% of piglets can be met on the 42nd day, and only 27% of piglets can be met on the 49th day. At this time, the nutritional needs of piglets are relatively high. Dependence on breast milk is reduced, and nutrients in feed can be taken in. Therefore, piglets can be weaned at 28 to 31 days of age, and the weaning weight can reach 6 to 7 kg (Wang 2021).

Practice has proved that the ideal way to reduce piglet stress is to maintain the original feed. A better weaning effect can be achieved by improving the feeding environment and nutrient supply. 1-2 days after weaning, the piglets are extremely unstable, and often roar, and it is night again. Therefore, in order to reduce the strong stress of piglets after weaning, it is generally necessary to ensure that the original pig house remains unchanged, and the principle of not changing the group or changing the house should be carried out. The weight should not exceed 2-3kg to prevent agitation and the piglets from biting each other. In addition, the piglets must be grouped according to their physiological status and gender, and the relatively weak piglets should be placed in one group. Group feeding of piglets helps to keep warm in winter, but it is not easy to have too many piglets in winter and spring. Especially in winter, it is necessary to carry out warm work, strengthen the piglet excretion at a fixed point, and ensure the health of the piglet (Wang 2021).

In the process of raising piglets, it is necessary to ensure that the piglets are on the basis of maintaining the original nutrient supply. Change the feed scientifically and reasonably to reduce the stress in the process of changing the feed. The feeding amount should be controlled at about 50% of the normal feeding amount on the first day after weaning of piglets. Feeding of piglets should follow the principle of small quantity and many times. The number of feedings was controlled at 5 to 6 times a day, and then the feeding amount was gradually increased. If the piglet does not have any abnormal reaction after 7 days of weaning, it can be carried out normal feeding. If the piglets develop symptoms such as diarrhea, normal feeding should be resumed after the piglets have recovered. When changing the feed, there needs to be a certain transition of changing the feed, and the amount of the creep feed can be reduced by 20% every day to gradually transition to the nursery feed (Sun 2021). In order to increase feed intake and promote digestion and absorption, weaned piglets should be regularly fed cooked feed. After weaning, it is necessary to appropriately increase dietary fat, reduce protein and other nutrients, and improve the digestive adaptability of the small intestine. Appropriately increase the feed intake of piglets, and add some bean dregs, fish meal, etc. to the feed to improve the growth rate of

piglets and enhance the physique of piglets.

1.3. Post-weaning Diarrhea

Post-weaning diarrhea is one of the most common causes of morbidity which result in reducing growth performance in piglets (Madec 2000). During the first two weeks after weaning, the gastrointestinal tract of piglets undergoes a dynamic stress process, likely due to nutritional, psychological, environmental and physiological factors (Laine et al. 2008). Temperature is one of the main environmental factors that influences the growth and development of piglets. If piglets go beyond their upper critical temperature, it will lead to heat stress and reduce their growth performance (Collin, et al. 2002). In contrast, low ambient temperature not only affects growth performance, but also increases diarrhea rate of piglets (Balsbaugh, et al., 1986). Enteric colibacillosis, pathogenic strains of *Escherichia coli* (*E.coli*) in weaned piglets can frequently proliferate and result in serious diarrhea, growth retardation, and increased mortality (Fairbrother et al., 2005). As a consequence, it will produce serious economic losses. To promote growth and prevent infections, antibiotics are widely used as a primary additive to relieve symptoms of diarrhea in weaned piglets. Unfortunately, the extensive use of antibiotics in animal production systems for the purposes mentioned above has contributed to antibiotic resistance and development of drug-resistant bacteria, which have been identified in meat, milk, eggs, and colonizing the human digestive tract and have resulted in foodborne disease outbreaks (Hur et al., 2011; Manyi-Loh et al., 2018; Shazali et al., 2014). The reason is that resistant bacteria occur in the animal intestine due to rapid selection, high mutation rates, and frequent transfer of resistance genes to other formerly susceptible bacteria following administration of a feed-based antibiotic (Kareem et al., 2016; Mathew et al., 2002). Since the quest for safer and healthier meat was remarkably increased in recent time, the use of nutritional feed additives is considered as promising strategy to alleviate intestinal disturbances around weaning of piglets. In the EU, pig mortality can be as high as 17%. A substantial proportion of these losses can be associated with gastrointestinal infections (Lalles et al., 2007). Since January 1st 2006, in the EU, the use of antibiotic growth promoters (AGP) was prohibited in livestock to reduce the phenomenon of antibiotic resistance (Mark et al., 2003). Chinese governments have also legislated the ban of antibiotic growth promoters in 2020 (2020). Therefore, there is an urgent need to search for possible alternatives as non-antibiotic growth promoters and to prevent frequently occurring post-weaning diarrhea in pigs. Several kinds of additives have been used to ameliorate the negative impacts of removing antibiotics from the diets, including essential oils (Zeng et al. 2015), organic acids (Namkung 2004), probiotics (Barba-Vidal et al., 2018), prebiotics (Yang et al., 2020) and so on. Among them, prebiotics are the most commonly used as potential alternatives to antibiotics.

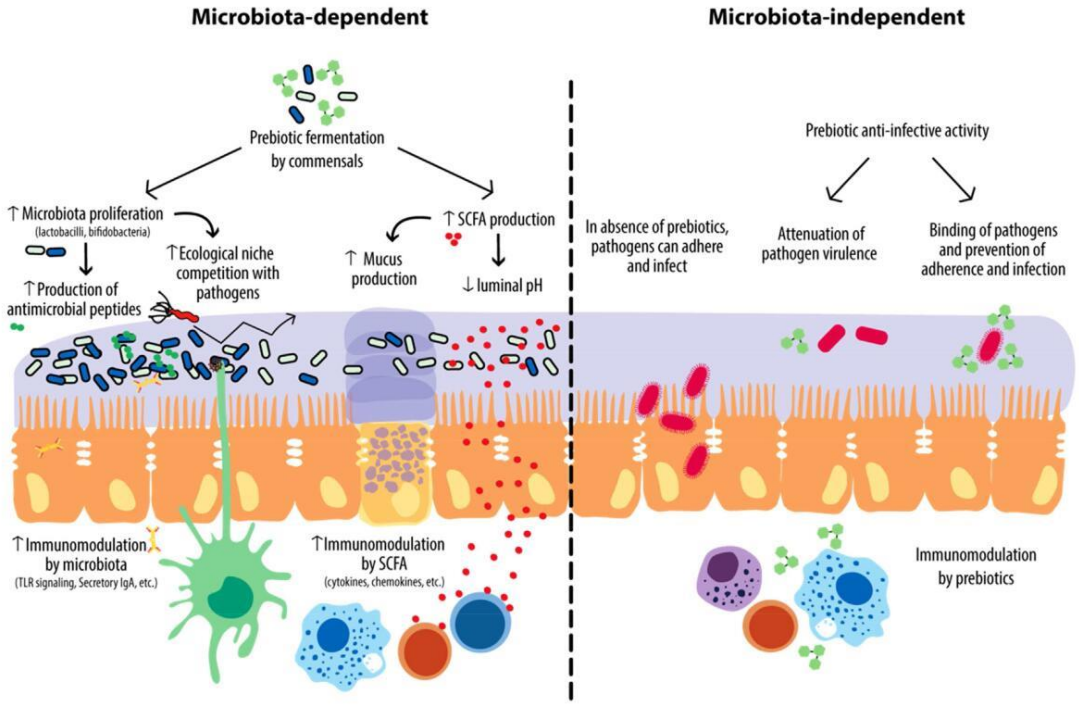


Figure 1-4. Prebiotics and their anti-infective mechanisms of action. Prebiotics exert their actions through both microbiota-dependent and microbiota independent mechanisms. IgA, immunoglobulin A; SCFAs, short-chain fatty acids; TLR, Toll-like receptor (Azagra-Boronat et al. 2019).

1.4. Prebiotics

The concept of "prebiotics" was initiated by Gibson and Roberfroid (Gibson and Roberfroid 1995). Originally, prebiotics are defined as "nondigestible food ingredients that selectively stimulate the composition and/or activity in the gastrointestinal beneficial microflora and thus considerably improves the host's health" (Gibson et al. 2004). This prebiotics concept was redefined several times. In 2015, Bindels et al. proposed the definition of prebiotics as "nondigestible compounds that, through their fermentation metabolism by microorganisms in the gut, modulate composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host." (Bindels et al. 2015). In 2017, the ISAPP proposed the following definition of a prebiotic: "a substrate that is selectively utilized by host microorganisms conferring a health benefit." (Gibson et al. 2017). Several studies have been carried out to establish their mechanism of action benefit exhibiting microbiota-dependent and microbiota-independent interactions (Figure 1-4). On the one hand, prebiotic compounds involve the stimulation of the composition and/or activity of the commensal bacteria found in the gut, eventually producing a broad array of effects that protect against pathogen colonization and preventing an overgrowth of undesired microbial communities. Therefore, the direct microbiota-dependent mechanism is one of the first barriers that pathogens encounter the gut (Kamada et al. 2013). Lewis et al. reported that prebiotic supplementation can decrease the recurrence of *C. difficile* diarrhea (Lewis et al. 2005). In addition, they increase the level of *Bifidobacterium* and *Lactobacillus* in the large intestine, which will induce intestinal epithelial cells to produce antimicrobial peptides targeting and killing invading pathogens (Gibson et al. 1995, Gibson et al. 2005, Jiao et al. 2014). Furthermore, prebiotics are fermented to short-chain fatty acids (SCFAs), which could reduce the gut pH and prevent the growth and colonization of

harmful microorganisms, such as some pathogenic species of bacteroides, clostridia, and coliforms in weaned piglets (Holscher 2017). Additionally, SCFAs have immunomodulatory properties which could bind to receptors on immune cells and inhibit histone deacetylases to enhance the host's defense (Vinolo et al. 2011). On the other hand, some compounds have also been considered as having novel microbiota-independent anti-infective activities and could involve direct interactions with pathogens and the immune system of host cells (Licht and Ebersbach 2012). Because of the prebiotic structures are associated with receptor mimicry, they can act as soluble receptor analogues, which will disrupt the microbial lectin-host receptor interactions, and dislodge the adherent pathogen from the intestinal epithelium (Shoaf-Sweeney et al. 2008). Such direct effects of carbohydrates are also associated with the modulation of virulence genes of enteric pathogens (Licht and Ebersbach 2012).

1.5. *Gut microbiota of pigs*

A healthy intestinal microbial community is diverse, stable, resistant and resilient. Several studies have shown that gut microbiota contributes significantly to maintenance of normal physiological and metabolic functioning of pigs. Several studies have showed dramatic changes microbial composition and huge diversity among different sections of intestine in the swine, with the most diverse group of microbes inhabiting in large intestine of pigs (Yang et al., 2016). Kim et al. and Zhao et al. found that *Proteobacteria* account for about 70% of the microbes in the jejunum and ileum, followed by *Firmicutes* which are about 20%. In contrast, in the cecum and colon, the *Firmicutes* ratio dominate with >75% and *Proteobacteria* are about 13% (Kim et al. 2012; Zhao et al. 2015). The structure and composition of the gut microbiota in pigs are determined by many factors, such as breed, age, diet and so on. For instance, in Chinese Jinhua pigs contain 70.4% *Firmicutes* of in the fecal bacterial population whereas 14.4% are *Bacteroidetes* (Yang et al. 2018). In contrast, western breeds such as the Duroc, Yorkshire, and Landrace, the gut microbiota is composed of 39.6%, 42.0%, and 45.6% *Firmicutes* and 57.0%, 51.4%, and 47.6% *Bacteroidetes*, respectively (Pajarillo et al. 2014a; 2015). One study compared the intestinal microbiota composition in Landrace pig (LD) and four Local Chinese pig Breeds (LCB) including Bama Mini-pig, Huanjiang mini-pig, Lantang pig and Ningxiang pig and reported that the LCBs have higher intestinal microbiota diversity than LD (Zhang, et al. 2014). In the pig intestine, the major taxa of microbiota are *Lactobacilli*, *Bifidobacterium*, *Streptococcus*, *Bacteroides*, *Clostridium perfringes*, and *Escherichia coli*; however, the specific composition changes with age (Dowarah et al. 2017). Petri et al. estimated that 34% of the total microbial population at 6h of birth is *Clostridiaceae*, which is seen to decrease to 1-13% by 20 days, while *Enterobacteriaceae* are not detected during the early period (Petri et al. 2010). Rather, *Enterobacteriaceae* increases steadily from weaning with 28 days to 5 days post-weaning; interestingly, they are seen to significantly decline after day 11 post-weaning (Dou et al. 2017*).* Diet remarkably impacts gut microbial diversity and is very important in maintaining health (Doré et al. 2015). The nutritional components of the animal diet can also be adjusted using different feed additives showing specific properties. Up to now, most of the nutritional studies conducted in weaning piglets have focused on increased feed intake, daily gain weight, improved immune function, or enhanced digestive functions and metabolism (Heo et al. 2012). Prebiotics have been shown to promote the growth of specific groups of commensal gastrointestinal microbiota and stimulate of the immune system, and improve the intestinal morphology and animal productivity. Therefore, it is important to modulate and change the

developmental program of the bacterial community and improve host health in the early life of weaned piglets. In this thesis, the prebiotic effects of XOS in weaned piglets were investigated. A thorough review on XOS is provided in chapter 2.

1.6. *The source, extraction method, chain length, purity and its impact of XOS on gut health.*

XOS is a well-known kind of functional oligosaccharide and extensively applied as a prebiotic. XOS have been found in some agricultural by-products, mainly including corncobs, wheat bran, sugarcane residues and rice straw (Jain et al., 2015; Samanta et al., 2015). The main preparation methods of XOS in present are by chemical, physical or enzymatic degradation methods (Chen et al., 2021). They are composed of xylose units linked by β-1, 4-xylosidic bonds, which have a branched structure by the addition of different side groups. XOS are sugar oligomers comprised of xylose units through β-(1-4)-xylosidic linkages; xylobiose (2 monomers), xylotriose (3 monomers), xylotetrose (4 monomers), xylopentose (5 monomers), xylohexose (6 monomers) and so on (Aachary and Prapulla, 2011; Kumar & Satyanarayana, 2011). The purity of XOS is usually in a range of 30%-95%. To produce food-grade XOS, the autohydrolysis liquors have to be refined by removing both monosaccharides and non-saccharide compounds to obtain a concentrate with a XOS content as high as possible. The usual purity of commercial XOS lies in the range of 75% to 95% (Aachary et al., 2011). The concentration range of XOS used in some animal studies is 35%-50% (Liu et al., 2018; Yin et al., 2019). XOS has the highest stability at a pH range of 2.5–8 and exhibits high temperature resistance (Amorim et al., 2019). In 2015, XOS were evaluated by the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies requested by the European Commission, who concluded that XOS did not present any toxicity and could be used in common and organic feed. (Turck et al., 2018). XOS have been widely reported to improve gut microbiota communities, especially for enhancing some beneficial microbiota such as *Bifidobacteria* and *Lactobacillus* and reducing some harmful bacteria. Some previous study showed that XOS administration decreased fecal *Escherichia coli* and increased *Lactobacilli* (Liu et al., 2018). However, a previous study reported that dietary XOS markedly reduced the *Lactobacillus* level and increased the relative abundances of *Streptococcus* and *Turicibacter*. in contrast with the another study where *Lactobacillus* was reduced (Yin et al., 2019). Oral administration of XOS to rat and mice significantly increased the moisture content of faeces, total caecum weight and population of *bifidobacteria* in addition to reduced caecum pH (Chung, et al., 2002; Chan, et al., 2005). Both healthy and diabetic rats maintained on a diet containing either 5 or 10% XOS exhibited significant increase in caecal *bifidobacteria* and *lactobacilli* population (Gobinath et al., 2010).

2. References

Aachary, A.A., and Prapulla, S.G. (2011). Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. Compr. Rev. Food. Sci. F 10, 2-16.

Amorim, C., S. C. Silvério, K. L. J. Prather, and L. R. Rodrigues. 2019. From lignocellulosic residues to market: Production and commercial potential of xylooligosaccharides. Biotechnol Adv 37(7):107397-. doi: 10.1016/j.biotechadv.2019.05.003.

Azagra-Boronat, I., M. J. Rodríguez-Lagunas, M. Castell and F. J. Pérez-Cano (2019). Prebiotics for Gastrointestinal Infections and Acute Diarrhea. Dietary Interventions in Gastrointestinal Diseases: 179-191.

Balsbaugh, R., K., S. E. Curtis, et al. (1986). Cold resistance and environmental temperature

preference in diarrheic piglets. Journal of Animal Science.
Barba-Vidal, E., S.M. Martín-Orúe and L. Castillejos (2018). Review: Are we using probiotics correctly in post-weaning piglets Animal, 12(12): 2489-2498.
Bindels, L. B., N. M. Delzenne, P. D. Cani and J. Walter (2015). Towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol 12(5): 303-310.
Chan, S. H., Chung, Y. C., Chang, C. T., & Chan, K. C. (2005). Effect of oligosaccharides on fecal content and intestinal microflora in adult Sprague-Dawley rats. Taiwanese Journal of Agricultural Chemistry and Food Science, 42, 440–447.
Chen, Y., Y. Xie, K.M. Ajuwon, R. Zhong, T. Li, L. Chen, H. Zhang, Y. Beckers and N. Everaert, 2021. Xylo-oligosaccharides, preparation and application to human and animal health: A review. Front Nutr, 8: 731930.
Chung, Y. C., Hsieh, C. P., & Chan, Y. C. (2002). Effects of xylooligosaccharides on the intestinal properties of ICR mice. Taiwanese Journal of Agricultural Chemistry and Food Science, 40, 377–384.
Collin, A. , Vaz, M. J. , & Dividich, J. L. . (2002). Effects of high temperature on body temperature and hormonal adjustments in piglets. Reproduction Nutrition Development, 42(1), 45-53.
Doré J, Blottière H. (2015) The influence of diet on the gut microbiota and its consequences for health. Curr Opin Biotechnol. 32:195–199.
Dou S, Gadonna-Widehem P, Rome V, Hamoudi D, Rhazi L, Lakhal L, Larcher T, Bahi-Jaber N, PinonQuintana A, Guyonvarch A, et al. (2017) Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. PLoS One. 12 (1):e0169851.
Dowarah R, Verma AK, Agarwal N, Patel BHM, Singh P. (2017) Effect of swine based probiotic on performance, diarrhoea scores, intestinal microbiota and gut health of grower-finisher crossbred pigs. Livestock Sci. 195:74–79.
Ebersbach, T., J. B. Andersen, A. Bergström, R. W. Hutkins and T. R. J. R. i. m. Licht (2012). Xylo-oligosaccharides inhibit pathogen adhesion to enterocytes in vitro. 163(1): 22-27.
Fairbrother, J.M., E. Nadeau and C.L. Gyles (2005). Escherichia coli in postweaning diarrhea in pigs: An update on bacterial types, pathogenesis, and prevention strategies. Animal health research reviews, 6(1): 17-39.
Gibson, G. R., E. R. Beatty, X. Wang and J. H. Cummings (1995). "Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin." Gastroenterology 108(4): 975-982.
Gibson, G. R., R. Hutkins, M. E. Sanders, S. L. Prescott, R. A. Reimer, S. J. Salminen, K. Scott, C. Stanton, K. S. Swanson, P. D. Cani, K. Verbeke and G. Reid (2017). "Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics." Nat Rev Gastroenterol Hepatol 14(8): 491-502.
Gibson, G. R., A. L. McCartney and R. A. Rastall (2005). "Prebiotics and resistance to gastrointestinal infections." Br J Nutr 93 Suppl 1: S31-34.
Gibson, G. R., H. M. Probert, J. V. Loo, R. A. Rastall and M. B. Roberfroid (2004). "Dietary modulation of the human colonic microbiota: updating the concept of prebiotics." Nutr Res Rev 17(2): 259-275.
Gibson, G. R. and M. B. Roberfroid (1995). "Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics." J Nutr 125(6): 1401-1412.
Gobinath, D., Madhu, A. N., Prashant, G., Srinivasan, K., & Prapulla, S. G. (2010). Beneficial effect of xylooligosaccharides and fructooligosaccharides in streptozotocin-induced diabetic rats. British Journal of Nutrition, 104, 40–47.
Gresse, R., F. Chaucheyras-Durand, M. A. Fleury, T. Van de Wiele, E. Forano and S. Blanquet-Diot (2017). "Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health." Trends Microbiol 25(10): 851-873.
Heo, J.M. et al. (2012) Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control postweaning diarrhoea without using in-feed antimicrobial compounds. J. Anim. Physiol. Anim. Nutr. (Berl) 97, 207–237
Holscher, H. D. (2017). "Dietary fiber and prebiotics and the gastrointestinal microbiota." Gut Microbes 8(2): 172-184.

Hur J., Kim J.H., Park J.H., Lee Y.J., Lee J.H. (2011). Molecular and virulence characteristics of multi-drug resistant salmonella enteritidis strains isolated from poultry. Vet. J. 189:306-311.

Jain, I., Kumar, V., and Satyanarayana, T. (2015). Xylooligosaccharides: An economical prebiotic from agroresidues and their health benefits. *Indian J. Exp. Biol.* 53**,** 131-142. PMID: 25872243.

Jiao, L., Z. Song, Y. Ke, K. Xiao, C. Hu, B. J. A. F. S. Shi and Technology (2014). "Cello-oligosaccharide influences intestinal microflora, mucosal architecture and nutrient transport in weaned pigs." 195: 85-91.

Kamada, N., G. Y. Chen, N. Inohara and G. Núñez (2013). "Control of pathogens and pathobionts by the gut microbiota." Nat Immunol 14(7): 685-690.

Kareem, K.Y., T.C. Loh, H.L. Foo, H. Akit and A.A. Samsudin. (2016). Effects of dietary postbiotic and inulin on growth performance, igf1 and ghr mrna expression, faecal microbiota and volatile fatty acids in broilers. BMC Vet Res, 12(1): 163.

Kim B, Borewicz K, White BA, Singer RS, Sreevatsan S, Tu ZJ, Isaacson RE. (2012). Microbial shifts in the swine distal gut in response to the treatment with antimicrobial growth promoter, tylosin. Proc Natl Acad Sci U.S.A.109 (38):15485–15490.

Kumar, V., & Satyanarayana, T. (2011). Applicability of thermoalkali-stable and cellulose free xylanase from a novel thermohalo-alkaliphilic Bacillus haloduransin producing xyloligosaccharides. Biotechnology Letters, 33, 2279–2285.

Lallès, Jean-Paul, Bosi P , Smidt H , et al. （2007） Nutritional management of gut health in pigs around weaning[J]. Proc Nutr Soc 66(02):260-268.

Laine, T. M., T. Lyytikainen, M. Yliaho and M. J. A. V. S. Anttila (2008). "Risk factors for post-weaning diarrhoea on piglet producing farms in Finland." 50(1): 1-11.

Lewis, S., S. Burmeister and J. Brazier (2005). "Effect of the prebiotic oligofructose on relapse of Clostridium difficile-associated diarrhea: a randomized, controlled study." Clin Gastroenterol Hepatol 3(5): 442-448.

Licht, T. R. and T. Ebersbach (2012). "Prebiotics for prevention of gut infections."

Liu, J., Cao, S., Liu, J., Xie, Y. and Zhang, H. (2018). Effect of probiotics and xylo-oligosaccharide supplementation on nutrient digestibility, intestinal health and noxious gas emission in weanling pigs. Asian-Australas J Anim Sci. 31(10): 1660-1669.

Madec, F. (2000). "Experimental models of porcine post-weaning colibacillosis and their relationship to post-weaning diarrhoea and digestive disorders as encountered in the field." Veterinary Microbiology 72(2000): 295-310.

Manyi-Loh C., Mamphweli S., Meyer E., Okoh A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. Molecules 23:795.

Mark, C., F. Christian, M. Enric, M.M. Paul and P. Ian, 2003. The european ban on growth-promoting antibiotics and emerging consequences for human and animal health. Journal of Antimicrobial Chemotherapy(2): 159-161.

Mathew A.G., Jackson F., Saxton A.M. (2002). Effects of antibiotic regimens on resistance of Escherichia coli and Salmonella servovar typhimurium in swine. J. Swine Health Prod. 10(1):7-13.

Ministry of Agriculture and Rural Affairs of the People's of Republic of China in 2020, No 194.

Namkung, H. L. J. G., M.， Yu, H.， Cottrill, M.， de Lange, C. F. M. (2004). "Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs %J The Canadian veterinary journal. La revue veterinaire canadienne." The Canadian veterinary journal 84(4): 697-704.

Pajarillo EAB, Chae JP, Balolong MP, Kim HB,Seo K-S, Kang D-K. (2014a) Pyrosequencing-based analysis of fecal microbial communities in three purebred pig lines. Journal of Microbiology. 52(8):646–651.

Pajarillo EAB, Chae JP, Balolong MP, Kim HB, Seo K-S, Kang D-K. (2015) Characterization of the fecal microbial communities of Duroc pigs using 16S rRNA gene pyrosequencing. Asian-Australas J Anim Sci. 28:584+.

Petri D, Hill JE, Van Kessel AG. (2010) Microbial succession in the gastrointestinal tract (GIT) of the preweaned pig. Livestock Sci. 133(1):107–109.

Popescu, A., 2020. Pork market crisis in romania: Pig livestock, pork production, consumption,

import, export, trade balance and price. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development.

Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., and Senani, S., et al. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: production and applications. Bioactive Carbohydrates and Dietary Fibre, 5(1), 62-71.

Shazali, N., H.L. Foo, T.C. Loh, D.W. Choe and R. Abdul Rahim. (2014). Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in malaysia. Gut Pathog, 6(1): 1.

Shoaf-Sweeney, K. D., R. W. J. A. i. f. Hutkins and n. research (2008). "Adherence, anti-adherence, and oligosaccharides: preventing pathogens from sticking to the host." 55: 101-161.

Sun, J. (2021) Physiological characteristics and feeding management techniques of weaned piglets. Modern Animal Husbandry Science and Technology, 1(73): 27-28.

Sun, R. (2021). “Feeding and management measures of weaned piglets.” China Animal Health, 17(3):106-107.

Turck, D., J. L. Bresson, B. Burlingame, T. Dean, S. Fairweather-Tait, M. Heinonen, K. I. Hirsch-Ernst, I. Mangelsdorf, H. J. Mcardle, and A. Naska, et al. 2018. Safety of xylooligosaccharides (XOS) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 16(7).

Vinolo, M. A., H. G. Rodrigues, R. T. Nachbar and R. Curi (2011). "Regulation of inflammation by short chain fatty acids." Nutrients 3(10): 858-876.

Wang, T. (2021) Technical points of feeding and management of weaned piglets. The Chinese Livestock and Poultry Breeding, 9 :173.

Wu, S., 2018. Analysis on the amount and utilization of manure in livestock and poultry breeding in china. Strategic Study of Chinese Academy of Engineering, 20(5): 103-111.

Yang, H., X. Huang, S. Fang, W. Xin, L. Huang and C. Chen, (2016). Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. Sci Rep, 6: 27427.

Yang, J., C. Wang, L. Liu and M. Zhang. (2020). *Lactobacillus reuteri* kt260178 supplementation reduced morbidity of piglets through its targeted colonization, improvement of cecal microbiota profile, and immune functions. Probiotics Antimicrob Proteins, 12(1): 194-203.

Yin, J., Li, F., Kong, X., Wen, C., Guo, Q., Zhang, L., et al. (2019). Dietary xylo-oligosaccharide improves intestinal functions in weaned piglets. Food Funct.

Zeng, Z., X. Xu, Q. Zhang, P. Li, P. Zhao, Q. Li, J. Liu and X. S. J. A. S. J. Piao (2015). "Effects of essential oil supplementation of a low-energy diet on performance, intestinal morphology and microflora, immune properties and antioxidant activities in weaned pigs." 86(3): 279-285.

Zhang, Z., 1993. A preliminary exploration of the history of pig farming in China. Agricultural Archaeology (01): 210-213.

Zhao W, Wang Y, Liu S, Huang J, Zhai Z, He C, Ding J, Wang J, Wang H, Fan W, et al. (2015). The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. PLoS One. 10 (2):e0117441.

2

Xylo-Oligosaccharides, Preparation and Application to Human and Animal Health: A Review

Background

This chapter briefly reviews preparation methods for xylo-oligosaccharides, and also discusses the current application of XOS to human and animal health. This review will open a new perspective on XOS potential applications for human consumption and animal production.

Article 1

Xylo-Oligosaccharides, Preparation and Application to Human and Animal Health: A Review

Yuxia Chen[1,2], Yining Xie[3], Kolapo M. Ajuwon[4], Ruqing Zhong[1], Tao Li[5], LiangChen[1], Hongfu Zhang[1]*, Yves Beckers[2], Nadia Everaert[2]*

[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China; yuxiatianya0428@126.com

[2]Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, Liège University, Passage des Déportés 2, Gembloux, Belgium

[3]School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, China

[4]Departments of Animal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A

[5]Hunan United Bio-technology Co., Changsha 410007, China

***Correspondence:**

chenliang01@caas.cn, zhanghongfu@caas.cn; Tel.: +86-10-6281-8910

This chapter was published in the *Frontiers in Nutrition.* (2021) 8:731930. doi: 10.3389/fnut.2021.731930

Abstract

Xylo-oligosaccharides (XOS) are considered as functional oligosaccharides and have great prebiotic potential. XOS are the degraded products of xylan prepared via chemical, physical or enzymatic degradation. They are mainly composed of xylose units linked by β-1, 4 bonds. XOS not only exhibit some specific physicochemical properties such as excellent water solubility and high temperature resistance, but also have a variety of functional biological activities including anti-inflammation, antioxidative, antitumor, antimicrobial properties and so on. Numerous studies have revealed in the recent decades that XOS can be applied to many food and feed products and exert their nutritional benefits. XOS have also been demonstrated to reduce the occurrence of human health-related diseases, improve the growth and resistance to diseases of animals. These effects open a new perspective on XOS potential applications for human consumption and animal production. Herein, this review aims to provide a general overview of preparation methods for XOS, and will also discuss the current application of XOS to human and animal health field.

Keywords: xylo-oligosaccharides, preparation, application, human health, animal health

1. Introduction

During the few last decades, there is increasing interest in the use of nutraceuticals or functional food additives for improving human health which has led to development of new food and feed products during the last few decades (Bitzios et al., 2011). Many functional products, having prebiotic characteristics, such as xylo-oligosaccharides (XOS), fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), chitooligosaccharides (COS), alginate-oligosaccharides (AOS) have been extensively used as food and feed additives (Samanta et al., 2015; Bali et al., 2015; Canfora et al., 2017; Yuan et al., 2019; Liu et al., 2019). Among these prebiotics, XOS are considered to be very promising. XOS are the degraded products prepared by chemical, physical or enzymatic degradation of xylan derived from biomass materials such as sugarcane residues, corn cobs, rice straw, etc (Jain et al., 2015, Figure 2-1). They are composed of xylose units linked by β-1, 4-xylosidic bonds, which have a branched structure by the addition of different side groups (Moreira et al.). The degrees of polymerization of XOS are usually 2–7 (Figure 2-2) and they are known as xylobiose, xylotriose, and so on (Aachary and Prapulla, 2011).

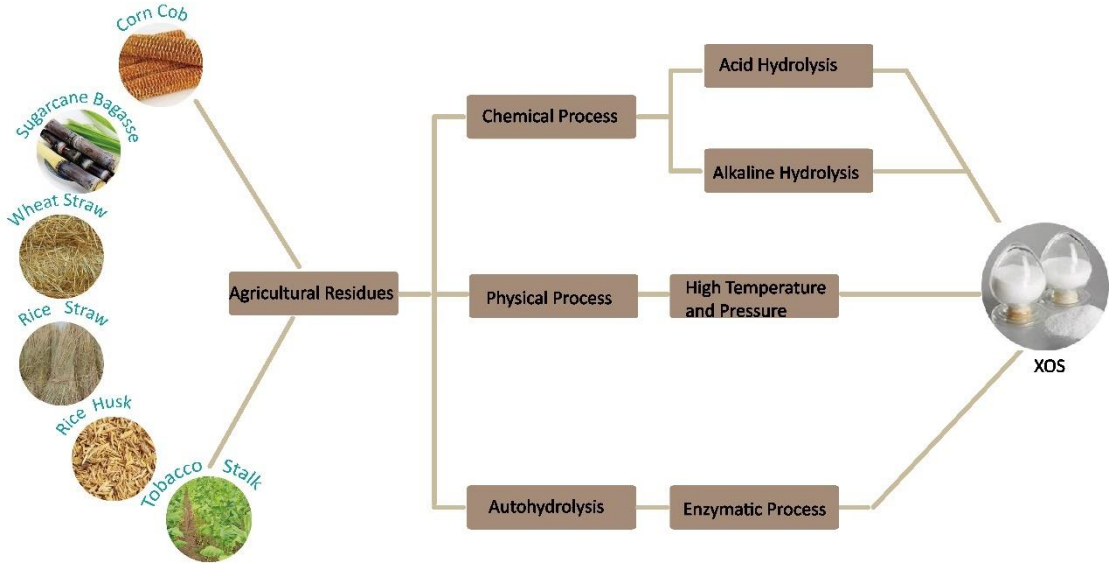


Figure 2-1. Schematic of XOS production from agricultural residues.

XOS have a high potential to be applied for human nutrition due to its physicochemical properties such as low viscosity, high water solubility, tolerance to high temperature and acidic pH (Wei et al., 2018). Studies shown that XOS display a variety of pharmacological activities, including anti-inflammation, antioxidative, antitumor, antimicrobial properties. In addition, XOS have a potential application in the animal husbandry (Ding et al., 2018; Liu et al., 2018). This review aims to summarize the methods of preparation of XOS and discuss the application of XOS to human and animal health.

2. Preparation and Characterization of XOS

The most widely used preparation methods of XOS are: (1) chemical degradation methods (2) physical degradation methods and (3) enzymatic degradation methods (Figure 2-3).

2.1. Chemical Process for the Production of XOS

The chemical degradation process, especially the acid and the alkaline hydrolysis methods,

has been widely used for the mass production of XOS in industry due to its advantages such as simple operation and low production cost. Several studies have been conducted on producing XOS with various inorganic acids (Samanta et al., 2012; Bian et al., 2014; Zhang et al., 2017; Zhang et al., 2017; Samanta et al., 2019). Samanta et al. reported that the xylan from tobacco stalks was hydrolysed by tartaric acid into XOS, mainly including xylobiose and xylotriose, in addition to monomeric xylose (Samanta et al., 2019). XOS production can also be obtained from corn cob xylan using weak sulphuric acid at 90 °C during 30 min (Samanta et al., 2012). The production of XOS depends on both acid concentration and hydrolysis time. A previous study showed that optimization of XOS production from waste xylan optimized by an orthogonal design of experiments, concluding a good extraction procedure of 20 min with 20% acetic acid at 140°C. A maximum XOS yield of more than 45.86% was obtained (Zhang et al., 2017). Ying et al. studied that the increment of sulfuric acid concentration promoted the yield of xylooligosaccharides from hydrogen peroxide-acetic acid-pretreated poplar from 0.69% to 20.45% (Ying et al., 2021). In addition, Zhang et al. reported that acetic acid hydrolysis provided the highest XOS yield, up to 45.91% compared to hydrochloric acid and sulfuric acid pretreatment (Zhang et al., 2017). It is widely known that the alkali solution could degrade hemicelluloses. This destruction is caused by the disruption of the hydrogen bonds with the alkaline reagent (de Freitas et al., 2019). In order to enhance the xylan content recovery from hemicellulose, use of appropriate alkaline concentration and pretreatment parameters are the primary conditions (Jnawali et al., 2017). For example, the use of higher concentration of alkali solution (15%) for extracting pineapple peels led to maximum recovery of hemicellulose. In the case of corn cobs, Samanta et al. also documented that higher concentration of alkali produced greater dissolution of hemicelluloses (Samanta et al., 2012). However, these methods caused corrosion of the equipment, thus limiting their use.

2.2 Physical Process for the Production of XOS

Production XOS products by physical degradation is relatively simple and environmentally friendly compared to chemical degradation. For example, XOS can be obtained from milled aspen wood using a microwave oven, processing at 180 °C for 10 min were and nextly subjected to fractionation to oligo- and polysaccharides by size-exclusion chromatography. The dispersion degree was smaller while the degradation effect was better (Teleman et al., 2000). The hydrothermal reactor can also be used to degrade the xylan. Its fragments released from corn cob hemicellulose are partially acetylated, which improves solubility of long xylo-oligosaccharides by preventing molecular interactions between the xylan and the main chains of the xylo-oligosaccharide and also by preventing the binding of xylan to cellulose. (Arai et al., 2019). The purity of XOS products is relatively high from physical degradation. However, there is limitation on the use of this method for large-scale production of XOS due to low yield.

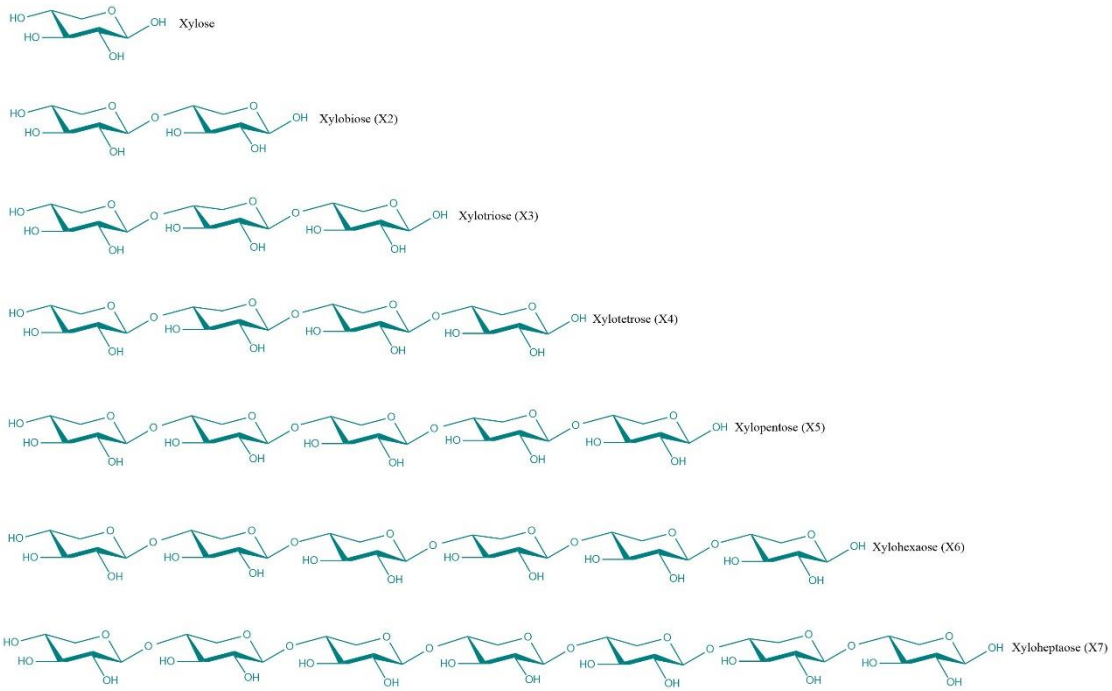


Figure 2-2. Chemical schematic structure of XOS with low degree of polymerization.

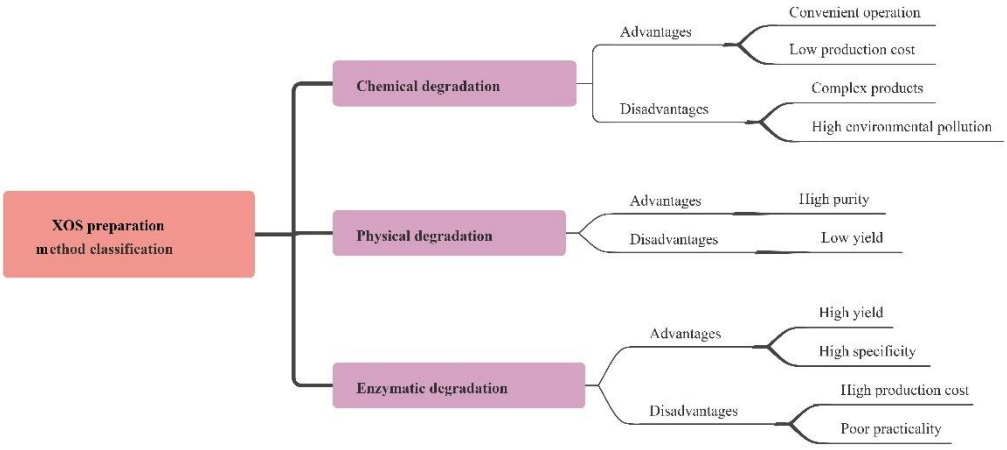


Figure 2-3. Characterization of XOS preparation methods.

2.3 Enzymatic Process for the Production of XOS

The industrial process of XOS production from natural xylan-rich agricultural residues involve enzymatic hydrolysis. As compared to the acid and alkaline hydrolysis method, production by the enzymatic degradation is relatively more economical, quick, and eco-friendly. Furthermore, enzymatic hydrolysis neither requires any special equipment nor produces undesirable byproducts. Thus, the production of XOS by enzymatic means was done from plant sources rich in xylan including corn cobs, sugarcane bagasse, wheat bran, birch wood, oat spelt, beech wood, natural grass, oil palm frond etc. These major enzymes used include β-xylosidase, glycosynthases and endo-xylanases, the latter being the key enzyme to produce XOS from xylan. They are able to reduce monomeric xylose release from the non-reducing ends of xylooligomers and xylobiose. The endo-xylanases from families GH10, GH11 and GH30 act specifically on the substituted and unsubstituted regions of xylan chain (Linares-Pasten et al.,

2018). Other studies focused on the use of β-xylosidases and glycosynthases for XOS production. β-xylosidases catalyze subtrate hydrolysis by inversion or retaining mechanism and are classified into six GH (glycoside hydrolase) families: GH3, 30, 39, 43, 52 and 54. The β-xylosidases have been reported to produce longer β-XOS from β-1, 4 linkages or synthesize novel XOS (Kurakake et al.; Dilokpimol et al., 2011). Kim et al. documented that a glycosynthase derived from a retaining xylanase could synthesize a great variety of XOS (Kim et al., 2006). Many factors affect the yield of XOS from xylan such as the enzyme activity, the raw material, and incubation conditions including incubation pH, reaction time and temperature (Jnawali et al., 2017).

Table 3-1 summarizes the preparation process and the yields of XOS produced from xylan and xylan biomass by different approaches, often leading to high yields for several sources of substrates. Importantly, the prebiotic action of XOS requires a low degree of polymerization (DP) (Wei et al., 2018; de Freitas et al., 2019). Hence, there are still some parameters in the preparation process of XOS that need to be optimized, including the production of a low DP (DP of 2-7) and the achievement of a high purity. Therefore, research focuses on the combination and integration of the processes, testing different raw materials, extraction methods and enzymes to achieve an economically viable and health promoting product with an optimal production efficiency.

3. Application of XOS to Human Health

XOS were demonstrated to have various activities in human health such as inducing immune modulation, anti-tumor, antioxidant and anti-microbial effects (Figure 3-4).

3.1. Immune Modulation Effects of XOS

It is essential for protecting the host from diseases or repairing tissue injury to release inflammatory mediators (Durack and Glauser, 1996; Childs et al., 2014), and XOS are thus suggested to be an immunomodulator to prevent adverse immune-related conditions. Indeed, XOS were shown to have immunomodulatory effects by regulating expression of several proinflammatory mediators in vitro. XOS not only suppressed TNF-α, IL-1β, IL-6 and NO expression, but also triggered IL-10 production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells (Chen et al., 2012). XOS feeding significantly decreased expression of IL-1β and IFN-γ and attenuated systemic inflammation (Hansen et al., 2013). Moreover, the O-acetylated XOS derived from almond shells and their deacetylated derivatives exhibited immunomodulatory potential, based on a mitogenic rat thymocyte test (Nabarlatz et al., 2007). Finally, XOS combined with inulin attenuated the expression of IL-1β in the blood of healthy subjects fed a high-fat diet (Lecerf et al., 2012). Schematic presentation of XOS health benefits and their role in immune modulation are depicted in Figure 2-4.

Table 2-1. Summary of XOS preparation and yields in the most recent studies.

substrate	Pretreatment	Biocatalyst	Yxylan/biomass (%)	Yxos/biomass (%)	Yxos/xylan (%)	DP	Reference
corn cobs	acetic acid pH 2.7, 150 °C, 30 min		30.4%	13.97%	45.9%	X2-X6	(Zhang et al., 2017)
	Dilute acid followed by 135 °C for 30 min	Xylanase from Penicillium corylophilum P-3-31	34.8%	23.6%	67.7%	X2-X4	(Yang et al., 2005)
	pH 6.5 and 60 °C	Xylanase (PbXyn10A)	31.2%	23.4%	75%	X2-X4	(Liu et al., 2018)
	ultra-high pressure pretreatment	Streptomyces thermovulgaris TISTR1948 endoxylanase	33.4%	3.6%	10.7%	X2-X4	(Seesuriyachan et al., 2017)
	190 °C, 13 min	GH10 xylanase	29.9%	14.8%	49.4%		(Arai et al., 2019)
	5% (w/v) KOH, 90 °C for 1 h		38.8%	11.5%	29.6%	X2-X5	(Boonchuay et al., 2018)
Sugarcane Bagasse	Alkaline 10% (w/v) at room temperature overnight	endo-β-1,4-xylanase rHlxyn11A	10.5%	6.0%	57.4%	X2-X3	(Xue et al., 2016)

	15% (w/v) aqueous ammonia	β-xylosidase	28.40%	19.3%	68.0%	X2-X4	(Reddy and Krishnan, 2016)
	0.24M dilute H_2SO_4 90 C 31 min		33.5%	9.7%	29%	X2-X6	(Bian et al., 2014)
	5% gluconic acid hydrolysis(w/v) 60 min at 150 °C	cellulase	26.5%	14.1%	53.2%	X2-X6	(Zhou et al., 2019)
	10% acetic acid at 150 °C for 45 min	G. oxydans ATCC 621H	27.9%	10.9%	39.1%	X2-X6	(Zhou and Xu, 2019)
Wheat straw	2 % NaOH at 80 °C for 90min	The endoxylanase-variant K80R	8.4%	3.3%	39.8%	X2-X3	(Faryar et al., 2015)
	Hydrolysis at 50 °C and pH 5 for 5 h	β-1,4-endoxylanase			44%	X2-X3	(Romero-Fernandez et al., 2018)
	180 °C 40min	endo-β-1-4-xylanase	73%	23%	31.5%	X2-X3	(Huang et al., 2017)
rice straw	2% w/w sulfuric acid, 100 ℃, 0.5h		65.3%	18.2%	27.8%		(Sophonputtanap

							hoca et al., 2018)
rice husk	12% w/v NaOH, 110–120 °C for 30 min	β-1,4-xylanase	54.5%	9.5%	17.4%	X2-X5	(Khat-Udomkiri et al., 2018)
pineapple peel	15% (w/v) alkali solution for 16 h at 45 °C,	endo- 1, 4--Xylanase M1		23.5%	25.7%	X2-X3	(Banerjee et al., 2019)
finger millet seed coat	50 °C, pH 5.0 and 15 U enzyme dose sodium acetate	xylanase of Thermomyces lanuginosus	4.8%	3.4%	71.8%	X2-X3	(Palaniappan et al., 2017)
tobacco stalk	8% KOH or NaOH 90°C, 1M tartaric acid		17.0%	6.1%	35.7%	X1-X3	(Samanta et al., 2019)

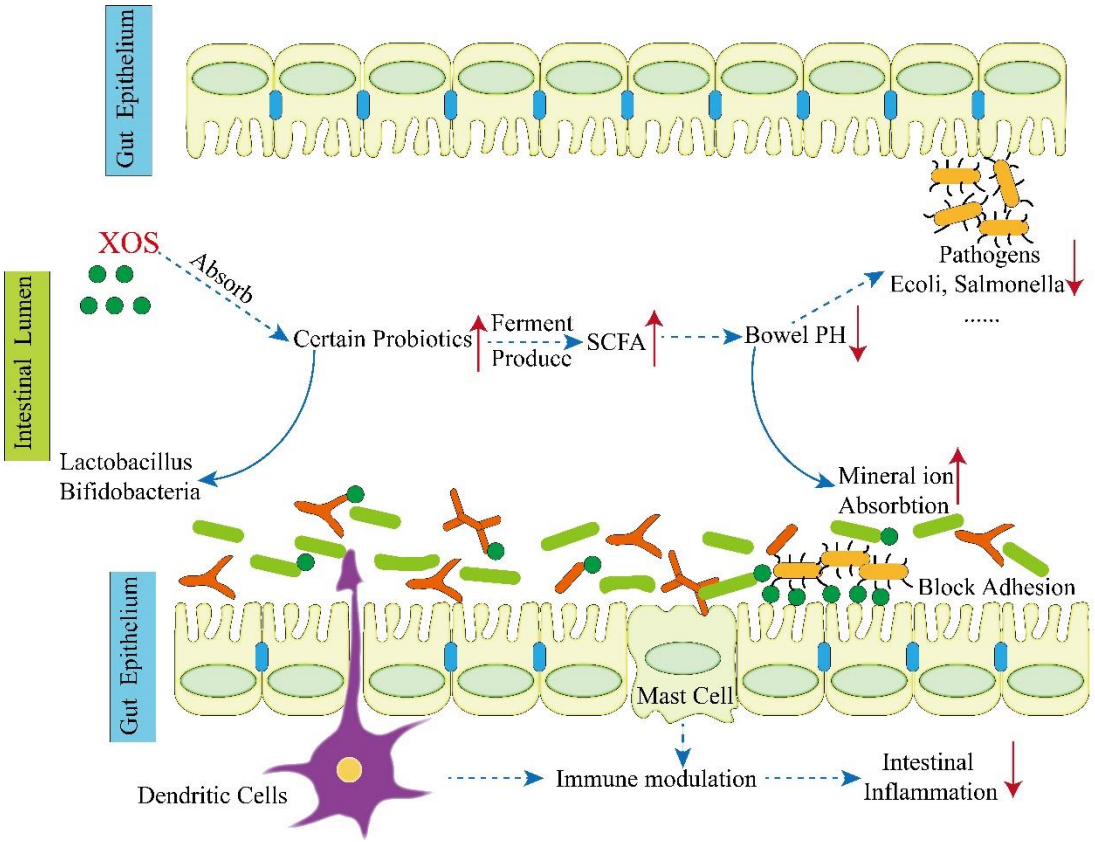


Figure 2-4. Potential health benefits of prebiotics (Aachary et al. 2009).

3.2. Anti-tumor Effects of XOS

The main causes of cancer are the uncontrolled proliferation of abnormal cells which may stay at the point of mutation or metastasize into other locations. It has been shown that XOS exposure showed effect in preventing cancer (Howe et al., 1992; Ando et al., 2004; Maeda et al., 2012). Indeed, β-1,3-Xylooligosaccharides with an average DP of 5 extracted from green alga Caulerpa lentillifera inhibited the number of viable human breast cancer MCF-7 cells in a dose-dependent manner, and induced apoptosis (Maeda et al., 2012). Thus, this XOS could be a promising agent for prevention of breast cancer. Moreover, XOS supplementation reduced the level of lipid peroxidation and increased the activities of glutathione-S-transferase and catalase in colonic mucosa and liver, which may have contributed to the inhibition of colon carcinogenesis (Aachary et al., 2015). In vitro approaches will be useful for future mechanistic characterization of the antitumor properties of XOS. However, no systematic attempts have been carried out to study the upstream signals of caspase activation and the specific effects in vivo. Further research is necessary to investigate the overall anti-tumor effect of XOS.

3.3. Antioxidant Effects of XOS

During both acute and chronic diseases in humans, the abundance of free radicals usually increases. Several notable studies demonstrated that XOS had exhibited strong antioxidant and free radical scavenging activity, thus suggesting a potential use in biomedical applications (Yu et al., 2015; Rashad et al., 2016). The scavenging ability of XOS was shown to be dose-dependent (Gowdhaman and Ponnusami, 2015), and this potential is likely attributable to efficient release of phenolic compounds and transfer of hydrogen atoms from the phenolic

compounds to free radicals (Huang et al., 2005). Jagtap et al. revealed that the percent of antioxidant activity gradually increased reaching the maximum, 74 % at a concentration of 6 mg/ml XOS using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, after which it did not show any further increase (Jagtap et al., 2017). Bouiche et al. studied that the antioxidant activity of glucuronoxylooligosaccharides (UXOS) and arabinoxylooligosaccharides (AXOS) was tested with the 2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method (Bouiche et al., 2019). The results showed that the antioxidant activity of UXOS was significantly higher than the antioxidant activity of AXOS. Although both have neutral molecules, UXOS also have methylglucuronic acid (MeGlcA) decorations that confer a negative charge to the XOS. It was assumed that the MeGlcA decorations of the XOS were key elements influencing their antioxidant and radical scavenging activity of XOS (Valls et al., 2018).

3.4. Anti-microbial Effects of XOS

It has been reported that XOS have significant antimicrobial effects against several pathogenic bacterial. A host of clinically important both Gram-negative and Gram-positive bacteria have been documented to be sensitive to XOS exposure. Indeed, XOS and FOS supplementations markedly reduced the cecal pH level and increased the population of bifidobacterial compared with the control and DMH (1,2-dimethylhydrazine) treatments and the XOS treatment group had a lower abundance of *E. coli* than the DMH group. These results indicated that XOS and FOS non-digestible carbohydrates may promote the health of intestinal tract (Hsu et al., 2004). In addition, some *in vitro* studies have documented that XOS supplementation produced lactic acid and acetic acid, which contributed to growth of *bifidobacteria* and *lactobacilli* strains and inhibited the growth of pathogenic strains (Palframan et al., 2003; Pan et al., 2009; Ebersbach et al., 2012; Dotsenko et al., 2017).

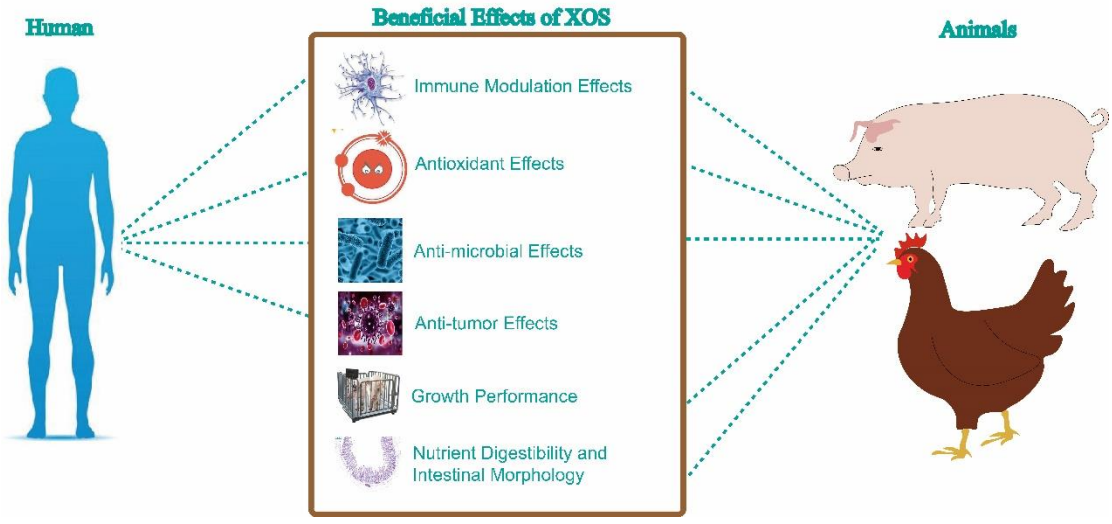


Figure 2-5. Health beneficial effects of XOS.

4. Application of XOS to Animal Health

In this section, the recent studies on the application of XOS in animal husbandry health are provided. We have noted that most of the studies were focusing on XOS modulation of growth performance, nutrient digestibility and intestinal morphology, immune and anti-oxidant activity and gut microbiome (Figure 2-5).

4.1. Effects of XOS on Growth Performance of Animals

XOS have been used for animal nutrition and health improvement due to their potential biological functions, such as, antioxidant, anti-inflammatory and antimicrobial effects. Previous studies have demonstrated the benefits of XOS on the growth performance of animals. Liu et.al reported that XOS treatment at a dose of 200 mg/kg increased average daily gain (ADG) by 17% and gain to feed (G/F) by 14% in the whole experiment, improved the apparent total tract digestibility (ATTD) of dry matter (DM), N and gross energy (GE) during 0 to 14 d in the piglets (Liu et al., 2018). Our study found that the effects of 500mg/kg XOS (XOS500) on the growth performance during 1 to 28 days were very similar with that of the antibiotic chlortetracycline in the piglets. The results showed that XOS500 (500mg/kg XOS) supplementation could significantly increase body weight (BW), ADG, average daily feed intake (ADFI) and feed to gain (F: G) of piglets (Chen et al., 2021a). However, another study failed to notice significant improvement on growth performance after 0.01% XOS treatment in pigs (Yin et al., 2019). The discrepancy might be caused by the different levels of XOS used in these studies. Thus, further studies are needed to confirm the optimal dose of XOS in pigs. In addition, Yuan et.al evaluated the effects of XOS on growth performance and immune function of broiler chickens. They reported that XOS supplementation in the diet of broiler chickens significantly improved ADFI and ADG at 1–42 days when compared to the control group (Yuan et al., 2018). The results of a study by Pourabedin et.al demonstrated that the feed conversion ratio (FCR) in broilers fed 2 g XOS/kg diet was lower than those fed 1 g XOS/kg diet between days 7 and 21, which is in line with other studies (Suo et al., 2015; De Maesschalck et al., 2015). Some other researchers found that the FCR in the control group was also significantly lower for the group receiving the XOS-supplemented diet in broiler chickens for the whole trial period (Suo et al., 2015; De Maesschalck et al., 2015). These results showed that XOS may dose-dependently improve the growth performance of animals and have potential as novel alternatives to antibiotics as growth promoters.

4.2. Effects of XOS on Nutrient Digestibility and Intestinal Morphology of Animals

The growth promoting effect of XOS has been shown to be related to improvement in nutrient digestibility. The addition of 200 mg/kg XOS with a purity of 50% supplementation has been demonstrated to improve the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and gross energy (GE) in weaning pigs on d14 (Liu et al., 2018). Similarly, the XOS supplementation significantly increased the apparent digestibility of the calcium with the increasing concentration of dietary XOS (0.1, 0.2, 0.3, 0.4 or 0.5 g/kg) in laying hens (Li et al., 2017). The improvement of nutrient digestibility may be the result from XOS supporting normal intestinal morphology. Intestine morphology indices are often as a useful criterion to estimate the nutrient digestion and absorption capacity of the intestine. It is generally believed that the jejunum is the main segment involved in absorption of nutrients and minerals (Schokker et al., 2017). Our study indicated that the XOS500 supplementation increased the villus height and villus height to crypt depth ratio in the jejunum and ileum in comparison with the CON and XOS1000 group in the piglets, possibly improving nutrient absorption (Chen et al., 2021b). Liu et.al confirmed that the XOS increased villus height to crypt depth ratio in jejunum, but did not influence villus height, crypt depth in the piglets (Liu et al., 2018). Similarly, Ding et.al reported that there was a linear improvement in villus height and villus height to crypt depth ratio of the jejunum as dietary XOS concentration increased in the laying hens (Ding et al., 2018). This is

in agreement with the study of Maesschalck et al. showing that supplementation of 0.5% XOS with a purity of 35% to broiler chicken feed significantly increased the villus height in the ileum, suggesting an increase in gut health and improved nutrient absorption (De Maesschalck et al., 2015). However, 0.01% XOS with a purity of 40% in the diet of weaned piglets had little effects on the intestinal structure and villus surface area (Yin et al., 2019). In addition, the addition of 75mg/kg XOS with a purity of 35% in the diet decreased the crypt depth of the duodenum (Suo et al., 2015). These results indicated that the use of an appropriate level of XOS may be important for increasing intestinal health and function.

4.3. Effects of XOS on Immune Modulation and Anti-oxidant Activity of Animals

XOS have been reported to display significant anti-inflammatory and anti-oxidant activities in animals in previous studies. In pigs, Yin et al. reported that dietary XOS markedly reduced serum IFN-γ concentration, indicating an anti-inflammatory effect of XOS (Yin et al., 2019), which is in line with a study in broilers showing a downregulation of the IFN-γ gene mRNA expression of jejunal mucosa. In addition, an increase in plasma IgG concentration was observed in XOS-fed 21-day-old broilers (Yuan et al., 2018). Furthermore, XOS increased plasma IgA, IL-2, and TNF-α concentration compared with the control diet, and linearly improved the IgA and TNF-α concentration in plasma increasing the dietary XOS concentration in the laying hens (Ding et al., 2018). These results indicated that dietary XOS may improve cell-mediated immune response in early weaned piglets by regulating the production of cytokines and antibodies. In addition, antioxidant defense systems are regarded as important serum indices for assessing animal health. The changes in the antioxidant defense systems mainly including total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) may indicate oxidative stress (Zhu et al., 2012). Several studies revealed that XOS had exhibited antioxidant and radical scavenging competency (Wang et al., 2011). However, the research of Guerreiro revealed that the XOS supplementation reduced antioxidant enzyme activities in European sea bass (Guerreiro et al., 2015).

4.4. Effects of XOS on the Modulation of Gut Microbiome of Animals

Our recent study showed that XOS500 supplementation could significantly increase the relative abundance of *Lactobacillus* genus and reduce the relative abundance of *Clostridium_sensu_stricto_1*, *Escherichia-Shigella*, and *Terrisporobacter* genus in the ileum and cecum in piglets (Chen et al., 2021a). Moreover, 200 mg/kg XOS administration decreased fecal *Escherichia coli* and increased *Lactobacilli* in piglets (Liu et al., 2018). However, dietary XOS reduced the relative abundance of the *Lactobacillus* and increased the relative abundances of *Streptococcus* and *Turicibacter* (Yin et al., 2019). Furthermore, XOS and GOS both markedly decreased the numbers of intestinal *Listeria* monocytogenes in ileal samples from guinea pigs, and selectively stimulated *bifidobacteria* and *lactobacilli*, which are believed to have inhibitory effects against pathogens (Ebersbach et al., 2010). Similar beneficial effects of XOS have been observed in broilers. Indeed, 2g XOS/kg diet increased the relative abundance of the *Lactobacillus* genus in the cecal microbiota of broilers (Pourabedin et al., 2015), that can adhere to the mucosa and epithelium, promoting colonization, immunomodulation and protecting the intestinal barrier against pathogens (Kravtsov et al., 2008). Furthermore, by the production of lactate, the lower the intestinal pH, inhibiting the growth of acid-sensitive pathogenic bacteria (Belenguer et al., 2007). However, the specific effect mechanism of XOS

on the gut microbiome remains unclear as several studies were only done (de Freitas 2019; Teleman et al., 2000; Jnawali et al., 2017) or by microbial culture methods (Arai et al., 2019) that fail to provide accurate taxonomic composition and community structure. Thus, extensive research will be required to determine effects of XOS on the microbiome in animals.

5. CONCLUSION

In this review paper, we have summarized the preparation methods for XOS and its potential use as a functional food or feed additive for human and animal health. XOS seem to beneficially promoting intestinal health by selective stimulation of growth of *bifidobacteria* and *lactobacilli*. XOS also reduce the abundance of potentially pathogenic organisms. In addition, XOS exhibit a variety of biological activities including effects in suppressing inflammation, antioxidative, antitumor and antimicrobial properties. However, there are still several bottlenecks in the preparation and application of XOS. It is still difficult to obtain XOS products in large scale with high purity, and lack of consistency in quality of different batches of XOS from different polymerization degrees due to a lack of standardized preparation methods. The XOS products in the market are mainly mixtures not monomers. Technologies should be developed for producing XOS monomers with high purity at low cost. In addition, new investigations are required to further elucidate the specific molecular mechanisms of XOS. Additional information is needed on the mode of absorption of XOS in the host after oral ingestion, and the identification of related receptors or responsible for the transportation of XOS into target cells. Progress in these areas may enhance the value of XOS for applications in the prevention and treatment of human diseases and animal production.

6. References

Aachary AA. 2009. Prebiotics: specific colonic nutrients. In: Bioactive xylooligosaccharides from corncob: enzymatic production and applications (Thesis) submitted to Univ. of Mysore, p. 19.

Aacharya, A. A., Gobinatha,D., Srinivasanb, K., and Prapulla, S. G. (2015). Protective effect of xylooligosaccharides from corncob on 1,2-dimethylhydrazine induced colon Cancer in rats [J]. Bioact. *Carbohydr. Diet. Fibre* 5(2):146-152. doi: 10.1016/j.bcdf.2015.03.004

Aachary, A.A., and Prapulla, S.G. (2011). Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. *Compr. Rev. Food. Sci. F* 10**,** 2-16. doi: 10.1111/j.1541-4337.2010.00135.x

Ando, H., Ohba, H., Sakaki, T., Takamine, K., Kamino, Y., Moriwaki, S., Bakalova, R., Uemura, Y., and Hatate, Y. (2004). Hot-compressed-water decomposed products from bamboo manifest a selective cytotoxicity against acute lymphoblastic leukemia cells. *Toxicol. In. Vitro.* 18**,** 765-771. doi: 10.1016/j.tiv.2004.03.011

Arai, T., Biely, P., Uhliarikova, I., Sato, N., Makishima, S., Mizuno, M., Nozaki, K., Kaneko, S., and Amano, Y. (2019). Structural characterization of hemicellulose released from corn cob in continuous flow type hydrothermal reactor. *J. Biosci. Bioeng.* 127**,** 222-230. doi: 10.1016/j.jbiosc.2018.07.016

Bali V., Panesar PS., Bera MB., and Panesar R. Fructo-oligosaccharides: (2015) Production, Purification and Potential Applications. Crit Rev Food Sci Nutr. 55(11):1475-90. doi: 10.1080/10408398.2012.694084

Banerjee, S., Patti, A.F., Ranganathan, V., and Arora, A. (2019). Hemicellulose based biorefinery from pineapple peel waste: Xylan extraction and its conversion into xylooligosaccharides. *Food. Bioprod. Process*. 117**,** 38-50. doi:10.1016/j.fbp.2019.06.012

Belenguer, A., Duncan, S.H., Holtrop, G., Anderson, S.E., Lobley, G.E., and Flint, H.J. (2007). Impact of pH on lactate formation and utilization by human fecal microbial communities. *Appl. Environ.. Microbiol.* 73**,** 6526-6533. doi: 10.1128/AEM.00508-07

Bian, J., Peng, P., Peng, F., Xiao, X., Xu, F., and Sun, R.-C. (2014). Microwave-assisted acid hydrolysis to produce xylooligosaccharides from sugarcane bagasse hemicelluloses. *Food. Chem.* 156**,** 7-13. doi: 10.1016/j.foodchem.2014.01.112

Bitzios, M., Fraser, I., and Haddock-Fraser, J. (2011). Functional ingredients and food choice: Results from a dual-mode study employing means-end-chain analysis and a choice experiment. *Food Policy*. 36**,** 715-725. doi: 10.1016/j.foodpol.2011.06.004

Boonchuay, P., Techapun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., Watanabe, M., Takenaka, S., and Chaiyaso, T. (2018). An integrated process for xylooligosaccharide and bioethanol production from corncob. *Bioresour. Technol.* 256**,** 399-407. doi: 10.1016/j.biortech.2018.02.004

Bouiche, C., Boucherba, N., Benallaoua, S., Martinez, J., Diaz, P., Pastor, F.I.J., and Valenzuela, S.V. (2019). Differential antioxidant activity of glucuronoxylooligosaccharides (UXOS) and arabinoxylooligosaccharides (AXOS) produced by two novel xylanases. *Int J Biol Macromol*. doi: 10.1016/j.ijbiomac.2019.11.073.

Canfora, E. E. , Van, D., Hermes, G., Goossens, G. H., Jocken, J., and Holst, J. J., et al. (2017). Supplementation of diet with galacto-oligosaccharides increases bifidobacteria, but not insulin sensitivity, inobeseprediabetic individuals. *Gastroenterology*. S0016508517354082. 10.1053/j.gastro.2017.03.051

Chen, Y., Xie, Y., Zhong, R., Liu, L., Lin, C., Xiao, L., Chen, L., Zhang, H., Beckers, Y., and Everaert, N. (2021a). Effects of Xylo-Oligosaccharides on Growth and Gut Microbiota as Potential Replacements for Antibiotic in Weaning Piglets. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.641172

Chen, Y., Xie, Y., Zhong, R., Han, H., Liu, L., Chen, L., and Zhang, H., et al. (2021b) Effects of graded levels of xylo-oligosaccharides on growth performance, serum parameters, intestinal morphology and intestinal barrier function in weaned piglets. *J. ANIM. SCI.* 99(7):skab183. doi: 10.1093/jas/skab183

Chen, H., Chen, Y., and Chang, H. (2012) Immunomodulatory Effects of Xylooligosaccharides[J]. *Food Sci. Technol. Res*. 18(2):195-199. doi: 10.3136/fstr.18.195

Childs, C.E., Roytio, H., Alhoniemi, E., Fekete, A.A., Forssten, S.D., Hudjec, N., et al.. (2014). Xylo-oligosaccharides alone or in synbiotic combination with Bifidobacterium animalis subsp. lactis induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. *Br. J. Nutr.* 111**,** 1945-1956. doi: 10.1017/S0007114513004261

De Freitas, C., Carmona, E., and Brienzo, M. (2019). Xylooligosaccharides production process from lignocellulosic biomass and bioactive effects. *Bioact. Carbohydr. Diet. Fibre* 18. doi: 10.1016/j.bcdf.2019.100184

De Maesschalck, C., Eeckhaut, V., Maertens, L., De Lange, L., Marchal, L., and Nezer, C., et al. (2015). Effects of Xylo-Oligosaccharides on Broiler Chicken Performance and

Microbiota. *Appl. Environ. Microbiol.* 81**,** 5880-5888. doi: 10.1128/AEM.01616-15

Dilokpimol, A., Nakai, H., Gotfredsen, C.H., Appeldoorn, M., Baumann, M.J., and Nakai, N., et al. (2011). Enzymatic synthesis of beta-xylosyl-oligosaccharides by transxylosylation using two beta-xylosidases of glycoside hydrolase family 3 from Aspergillus nidulans FGSC A4. *Carbohydr. Res.* 346**,** 421-429. doi: 10.1016/j.carres.2010.12.010

Ding, X.M., Li, D.D., Bai, S.P., Wang, J.P., Zeng, Q.F., and Su, Z.W., et al. (2018). Effect of dietary xylooligosaccharides on intestinal characteristics, gut microbiota, cecal short-chain fatty acids, and plasma immune parameters of laying hens. *Poult. Sci.* 97**,** 874-881. doi: 10.3382/ps/pex372

Dotsenko, G., Meyer, A.S., Canibe, N., Thygesen, A., Nielsen, M.K., and Lange, L. (2017). Enzymatic production of wheat and ryegrass derived xylooligosaccharides and evaluation of their in vitro effect on pig gut microbiota. *Biomass Convers. Bior.* 8**,** 497-507. doi: 10.1007/s13399-017-0298-y

Durack, D.T., and Glauser, M.P. (1996). The inflammatory cytokines - New developments in the pathophysiology and treatment of septic shock - Discussion. *Drugs* 52**,** 17-17. doi: 10.2165/00003495-199600522-00004

Ebersbach, T., Jorgensen, J.B., Heegaard, P.M., Lahtinen, S.J., Ouwehand, A.C., and Poulsen, M., et al. (2010). Certain dietary carbohydrates promote Listeria infection in a guinea pig model, while others prevent it. *Int. J. Food. Microbiol.* 140**,** 218-224. doi: 10.1016/j.ijfoodmicro.2010.03.030

Ebersbach, T., Andersen, J.B., and Bergstr, M. A., et al. (2012) Xylo-oligosaccharides inhibit pathogen adhesion to enterocytes in vitro[J]. *Res. Microbiol* 163(1):22-27. doi: 10.1016/j.resmic.2011.10.003

Faryar, R., Linares-Pastén, J.A., Immerzeel, P., Mamo, G., Andersson, M., and Stålbrand, H., et al. (2015). Production of prebiotic xylooligosaccharides from alkaline extracted wheat straw using the K80R-variant of a thermostable alkali-tolerant xylanase. *Food. Bioprod. Process.*. 93**,** 1-10. doi: 10.1016/j.fbp.2014.11.004

Gowdhaman, D., and Ponnusami, V. (2015). Production and optimization of xylooligosaccharides from corncob by Bacillus aerophilus KGJ2 xylanase and its antioxidant potential. *Int. J. Biol. Macromol.* 79**,** 595-600. doi: 10.1016/j.ijbiomac.2015.05.046

Guerreiro, I., Couto, A., Pérez-Jiménez, A., Oliva-Teles, A., and Enes, P. (2015). Gut morphology and hepatic oxidative status of European sea bass (Dicentrarchus labrax) juveniles fed plant feedstuffs or fishmeal-based diets supplemented with short-chain fructo-oligosaccharides and xylo-oligosaccharides. *Br. J. Nutr.* 114**,** 1975-1984. doi: 10.1017/S0007114515003773

Hansen, C.H., Frokiaer, H., Christensen, A.G., Bergstrom, A., Licht, T.R., and Hansen, A.K., et al. (2013). Dietary xylooligosaccharide downregulates IFN-gamma and the low-grade inflammatory cytokine IL-1beta systemically in mice. *J. Nutr.* 143**,** 533-540. doi: 10.3945/jn.112.172361

Howe, G.R., Benito, E., Castelleto, R., Cornée, J., Estève, J., and Gallagher, R.P., et al. (1992). Dietary intake of fiber and decreased risk of cancers of the colon and rectum: evidence from the combined analysis of 13 case-control studies. *Jnci-J. Natl. Cancer.* 84**,** 1887-1896. doi: 10.1093/jnci/84.24.1887

Hsu C. K., Liao J. W., Chung Y. C., Hsieh C. P., and Chan Y. C. (2004) Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats. *J. Nutr.* Jun;134(6):1523-8. doi: 10.1093/jn/134.6.1523. PMID: 15173423

Huang, C., Lai, C., Wu, X., Huang, Y., He, J., Huang, C., Li, X., and Yong, Q. (2017). An integrated process to produce bio-ethanol and xylooligosaccharides rich in xylobiose and xylotriose from high ash content waste wheat straw. *Bioresour Technol* 241**,** 228-235. doi: 10.1016/j.biortech.2017.05.109

Huang, D., Ou, B., and Prior, R.L. (2005). The chemistry behind antioxidant capacity assays. *J. Agric. Food. Chem.* 53**,** 1841-1856. doi: 10.1021/jf030723c

Jagtap, S., Deshmukh, R.A., Menon, S., and Das, S. (2017). Xylooligosaccharides production by crude microbial enzymes from agricultural waste without prior treatment and their potential application as nutraceuticals. *Bioresour Technol* 245**,** 283-288. doi: 10.1016/j.biortech.2017.08.174

Jain, I., Kumar, V., and Satyanarayana, T. (2015). Xylooligosaccharides: An economical prebiotic from agroresidues and their health benefits. *Indian J. Exp. Biol.* 53**,** 131-142. PMID: 25872243

Jnawali, P., Kumar, V., Tanwar, B., Hirdyani, H., and Gupta, P. (2017). Enzymatic Production of Xylooligosaccharides from Brown Coconut Husk Treated with Sodium Hydroxide. *Waste Biomass Valori*. doi: 10.1007/s12649-017-9963-4

Khat-Udomkiri, N., Sivamaruthi, B.S., Sirilun, S., Lailerd, N., Peerajan, S., and Chaiyasut, C. (2018). Optimization of alkaline pretreatment and enzymatic hydrolysis for the extraction of xylooligosaccharide from rice husk. *AMB. Express* 8**,** 115. doi: 10.1186/s13568-018-0645-9

Kim, Y.W., Fox, D.T., Hekmat, O., Kantner, T., Mcintosh, L.P., Warren, R.a.J., and Withers, S.G. (2006). Glycosynthase-based synthesis of xylo-oligosaccharides using an engineered retaining xylanase from Cellulomonas fimi. *Org. Biomol. Chem*. 4**,** 2025-2020. doi: 10.1039/b601667g

Kravtsov, E., Yermolayev, A., Anokhina, I., Yashina, N., Chesnokova, V., and Dalin, M. (2008). Adhesion characteristics of Lactobacillus is a criterion of the probiotic choice. *B. Exp. Biol. Med.* 145**,** 232-234. doi: 10.1007/s10517-008-0058-x

Kurakake, M., Fujii, T., Yata, M., Okazaki, T., and Komaki, T. (2005) Characteristics of transxylosylation by β-xylosidase from Aspergillus awamori K4. *Biochim. Biophys. Acta.* 1726**,** 0-279. doi: 10.1016/j.bbagen.2005.08.009

Lecerf, J.-M., Dépeint, F., Clerc, E., Dugenet, Y., Niamba, C.N., and Rhazi, L. et al. (2012). Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. *Br. J. Nutr.* 108**,** 1847-1858. doi: 10.1017/S0007114511007252

Li, D.D., Ding, X.M., Zhang, K.Y., Bai, S.P., Wang, J.P., and Zeng, Q.F., et al. (2017). Effects of dietary xylooligosaccharides on the performance, egg quality, nutrient digestibility and plasma parameters of laying hens. *Anim. Feed Sci. Tech.* 225**,** 20-26. doi: 10.1016/j.anifeedsci.2016.12.010

Linares-Pasten, J.A., Aronsson, A., and Karlsson, E.N. (2018). Structural Considerations on the Use of Endo-Xylanases for the Production of prebiotic Xylooligosaccharides from Biomass.

Curr. Protein Pept. Sci. 19**,** 48-67. doi: 10.2174/1389203717666160923155209

Liu, J., Cao, S., Liu, J., Xie, Y., and Zhang, H. (2018). Effect of probiotics and xylo-oligosaccharide supplementation on nutrient digestibility, intestinal health and noxious gas emission in weanling pigs. *Asian-Australas J. Anim. Sci.* 31**,** 1660-1669. doi: 10.5713/ajas.17.0908

Liu, J., Yang, S., Li, X., Yan, Q., and Jiang, Z. (2019). Alginate oligosaccharides: production, biological activities, and potential applications. *Compr Rev Food Sci Food Saf*. Nov;18(6):1859-1881. doi: 10.1111/1541-4337.12494

Liu, X., Liu, Y., Jiang, Z., Liu, H., Yang, S., and Yan, Q. (2018). Biochemical characterization of a novel xylanase from Paenibacillus barengoltzii and its application in xylooligosaccharides production from corncobs. *Food Chem.* 264**,** 310-318. doi: 10.1016/j.foodchem.2018.05.023

Maeda, R., Ida, T., Ihara, H., and Sakamoto, T. (2012). Induction of apoptosis in MCF-7 cells by beta-1,3-xylooligosaccharides prepared from Caulerpa lentillifera. *Biosci. Biotech. Bioch.* 76**,** 1032-1034. doi: 10.1271/bbb.120016

Nabarlatz, D., Montane, D., Kardosova, A., Bekesova, S., Hribalova, V., and Ebringerova, A. (2007). Almond shell xylo-oligosaccharides exhibiting immunostimulatory activity. *Carbohydr. Res.* 342**,** 1122-1128. doi: 10.1016/j.carres.2007.02.017

Palaniappan, A., Balasubramaniam, V.G., and Antony, U. (2017). Prebiotic potential of xylooligosaccharides derived from finger millet seed coat. *Food Biotechnol.* 31, 264-280. doi:10.1080/08905436.2017.1369433

Palframan, R.J., Gibson, G.R., and Rastall, R.A. (2003). Carbohydrate preferences of Bifidobacterium species isolated from the human gut. *Curr. Issues Intest. Microbiol.* 4, 71-75. PMID: 14503691

Pan, X., Wu, T., Zhang, L., Cai, L., and Song, Z. (2009). Influence of oligosaccharides on the growth and tolerance capacity of lactobacilli to simulated stress environment. *Lett. Appl. Microbiol.* 48**,** 362-367. doi: 10.1111/j.1472-765X.2008.02539.x

Pourabedin, M., Guan, L., and Zhao, X. (2015). Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. *Microbiome* 3, 15. doi: 10.1186/s40168-015-0079-4

Rashad, M.M., Mahmoud, A.E., Nooman, M.U., Mahmoud, H.A., and Keshta, A.T. (2016). Production of antioxidant xylooligosaccharides from lignocellulosic materials using Bacillus amyloliquifaciens NRRL B-14393 xylanase. *J.App.Pharm.Sci*. 6, 30-36. doi: 10.7324/JAPS.2016.60606

Reddy, S.S., and Krishnan, C. (2016). Production of high-pure xylooligosaccharides from sugarcane bagasse using crude β-xylosidase-free xylanase of Bacillus subtilis KCX006 and their bifidogenic function. *LWT -Food Sci. Technol.* 65**,** 237-245. doi: 10.1016/j.lwt.2015.08.013

Romero-Fernandez, M., Moreno-Perez, S., Martins De Oliveira, S., Santamaria, R.I., Guisan, J.M., and Rocha-Martin, J. (2018). Preparation of a robust immobilized biocatalyst of beta-1,4-endoxylanase by surface coating with polymers for production of xylooligosaccharides from different xylan sources. *N Biotechnol* 44**,** 50-58. doi: 10.1016/j.nbt.2018.04.007

Samanta, A., Chikkerur, J., Roy, S., Kolte, A., Sridhar, M., Dhali, A., Giridhar, K., and Senani, S. (2019). Xylooligosaccharides production from tobacco stalk xylan using edible acid.

Current Science (00113891) 117

Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., and Senani, S., et al. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: production and applications. Bioactive Carbohydrates and Dietary Fibre, 5(1), 62-71. doi: 10.1016/j.bcdf.2014.12.003

Samanta, A., Senani, S., Kolte, A.P., Sridhar, M., Sampath, K., and Jayapal, N., et al. (2012). Production and in vitro evaluation of xylooligosaccharides generated from corn cobs. *Food and Bioprod. Process.*, 466-474. doi: 10.1016/j.fbp.2011.11.001

Schokker, D., Jansman, A.J., Veninga, G., De Bruin, N., Vastenhouw, S.A., and De Bree, F.M., et al. (2017). Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. *BMC Genomics* 18**,** 241. doi: 10.1186/s12864-017-3625-6

Seesuriyachan, P., Kawee-Ai, A., and Chaiyaso, T. (2017). Green and chemical-free process of enzymatic xylooligosaccharide production from corncob: Enhancement of the yields using a strategy of lignocellulosic destructuration by ultra-high pressure pretreatment. *Bioresour Technol.* 241**,** 537-544. doi: 10.1016/j.biortech.2017.05.193

Sophonputtanaphoca, S., Pridam, C., Chinnak, J., Nathong, M., and Juntipwong, P. (2018). Production of non-digestible oligosaccharides as value-added by-products from rice straw. *Agric. Nat. Resour.* 52**,** 169-175. doi: 10.1016/j.anres.2018.06.013

Suo, H. Q., Lin, L., Xu, G. H., Xiao, L., Chen, X. G., and Xia, R. R., et al. (2015) Effectiveness of dietary xylo-oligosaccharides for broilers fed a conventional corn-soybean meal diet. J. Integr. Agr., 14(010):2050-2057. doi: 10.1016/S2095-3119(15)61101-7

Teleman, A., Lundqvist, J., Tjerneld, F., Stålbrand, H., and Dahlman, O. (2000). Characterization of acetylated 4-O-methylglucuronoxylan isolated from aspen employing 1H and 13C NMR spectroscopy. *Carbohydr Res.* 329(4):807-15. doi: 10.1016/s0008-6215(00)00249-4

Valls, C., Pastor, F.J., Vidal, T., Roncero, M.B., Díaz, P., Martínez, J., and Valenzuela, S.V. (2018). Antioxidant activity of xylooligosaccharides produced from glucuronoxylan by Xyn10A and Xyn30D xylanases and eucalyptus autohydrolysates. *Carbohydr. Polym.* 194**,** 43-50. doi: 10.1016/j.carbpol.2018.04.028

Wang, J., Cao, Y., Wang, C., and Sun, B. (2011). Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet. *Carbohydr. Polym.* 86**,** 1192-1197. doi: 10.1016/j.carbpol.2011.06.014

Wei, L., Yan, T., Wu, Y., Chen, H., and Zhang, B. (2018). Optimization of alkaline extraction of hemicellulose from sweet sorghum bagasse and its direct application for the production of acidic xylooligosaccharides by Bacillus subtilis strain MR44. *PLoS One* 13**,** e0195616. doi: 10.1371/journal.pone.0195616

Xue, J.L., Zhao, S., Liang, R.M., Yin, X., Jiang, S.X., and Su, L.H., et al. (2016). A biotechnological process efficiently co-produces two high value-added products, glucose and xylooligosaccharides, from sugarcane bagasse. *Bioresour. Technol.* 204**,** 130-138. doi: 10.1016/j.biortech.2015.12.082

Yang, R., Xu, S., Wang, Z., and Yang, W. (2005). Aqueous extraction of corncob xylan and production of xylooligosaccharides. *LWT -Food Sci. Technol.* 38**,** 677-682. doi: 10.1016/j.lwt.2004.07.023

Yin, J., Li, F., Kong, X., Wen, C., Guo, Q., and Zhang, L., et al. (2019). Dietary xylo-oligosaccharide improves intestinal functions in weaned piglets. *Food Funct* 10**,** 2701-2709. doi: 10.1039/c8fo02485e

Ying W., Xu, Y., Zhang, J. (2021) Effect of sulfuric acid on production of xylooligosaccharides and monosaccharides from hydrogen peroxide-acetic acid-pretreated poplar. *Bioresour Technol*. Feb;321:124472. doi: 10.1016/j.biortech.2020

Yu, X., Yin, J., Li, L., Luan, C., and Li, S. (2015). Prebiotic Potential of Xylooligosaccharides Derived from Corn Cobs and Their In Vitro Antioxidant Activity When Combined with Lactobacillus. *J. Microbiol Biotechnol*. doi: 10.4014/jmb.1501.01022

Yuan, L., Li, W., Huo, Q., Du, C., Wang, Z., Yi, B., and Wang, M. (2018). Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens. *PeerJ.* 6**,** e4435. doi: 10.7717/peerj.4435. eCollection 2018

Yuan X., Zheng J., Jiao S., Cheng G., Feng C., Du Y., and Liu H. (2019A). A review on the preparation of chitosan oligosaccharides and application to human health, animal husbandry and agricultural production. Carbohydr Polym. Sep 15;220:60-70. doi: 10.1016/j.carbpol.2019.05.050

Zhang, H., Xu, Y., and Yu, S. (2017). Co-production of functional xylooligosaccharides and fermentable sugars from corncob with effective acetic acid prehydrolysis. *Bioresour. Technol.* 234**,** 343-349. doi: 10.1016/j.biortech.2017.02.094

Zhang, H., Zhou, X., Xu, Y., and Yu, S. (2017). Production of xylooligosaccharides from waste xylan, obtained from viscose fiber processing, by selective hydrolysis using concentrated acetic acid. *J. Wood Chem. Technol*. 37**,** 1-9. doi: 10.1080/02773813.2016.1214154

Zhou, X., and Xu, Y. (2019). Integrative process for sugarcane bagasse biorefinery to co-produce xylooligosaccharides and gluconic acid. *Bioresour Technol* 282**,** 81-87. doi: 10.1016/j.biortech.2019.02.129

Zhou, X., Zhao, J., Zhang, X., and Xu, Y. (2019). An eco-friendly biorefinery strategy for xylooligosaccharides production from sugarcane bagasse using cellulosic derived gluconic acid as efficient catalyst. *Bioresour Technol* 289**,** 121755. doi: 10.1016/j.biortech.2019.121755

Zhu, L.H., Zhao, K.L., Chen, X.L., and Xu, J.X. (2012). Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. *J. Anim. Sci.* 90**,** 2581-2589. doi: 10.2527/jas.2012-4444

3

Objectives and structure of thesis

Objectives and Structure of the thesis

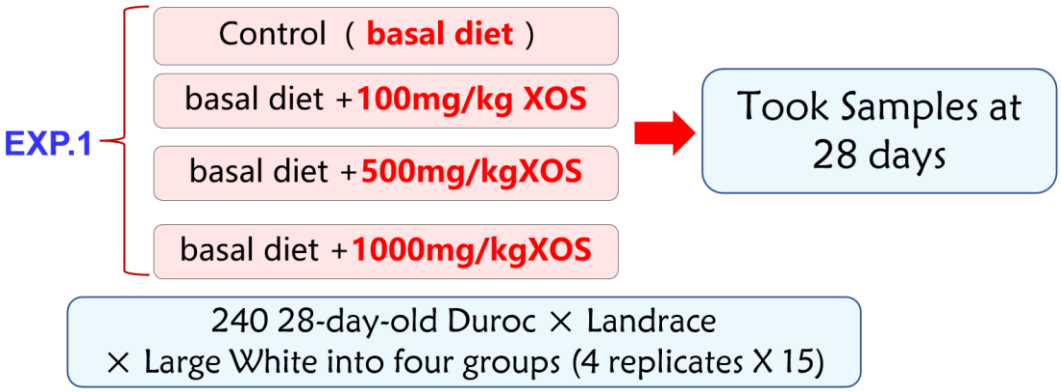
1. Objectives of the thesis

Weaning piglets in modern swine industry is often challenged by post-weaning stresses including dietary, social, and environmental changes. These stresses result in increasing disease and mortality risks such as post-weaning diarrhea. To promote animal growth and prevent infections, prebiotics from the agricultural by-products are nutrients that have the potential to considerably influence the physiology of the whole body and, consequently, health, and well-being. XOS are considered as functional oligosaccharides and have great prebiotic potential. However, the optimal XOS supplementation dosage used in the diet of weaned piglets is still unknown. This thesis aimed to investigate the optimal dose of XOS to be added in post-weaning diets, to positively affect growth performance, host physiology and intestinal functioning. Secondly, the aim was to investigate if XOS can replace antibiotics, by the determination of growth performance, microbiota and their main metabolites before and after weaning and therefore demonstrate its efficiency.

2. Structure of the thesis

To investigate the first aim, a dose-testing trial was performed (Figure 3.1, Exp 1). This part is described in chapter 4. This chapter was published in Journal of Animal Sciences. For the second aim, to investigate the potential of XOS as compared to an antibiotic, a second trial was performed (Figure 3.1, Exp 2). The XOS dose used in the second trial consisted of the optimal dose of the first trial (500 mg XOS/kg diet). This part is described in chapter 5, and was published in Frontiers in Microbiology. The technical route of the experiments performed during this thesis are represented in Figure 3.2. Finally, chapter 6 consists of the general discussion and perspectives.

Exp.1:



Exp.2:

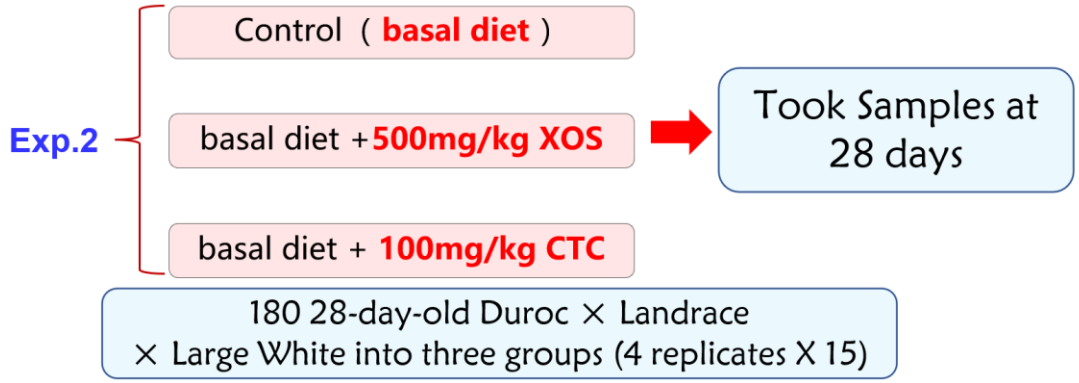


Figure 3-1: The contents of the two experiments during this thesis.

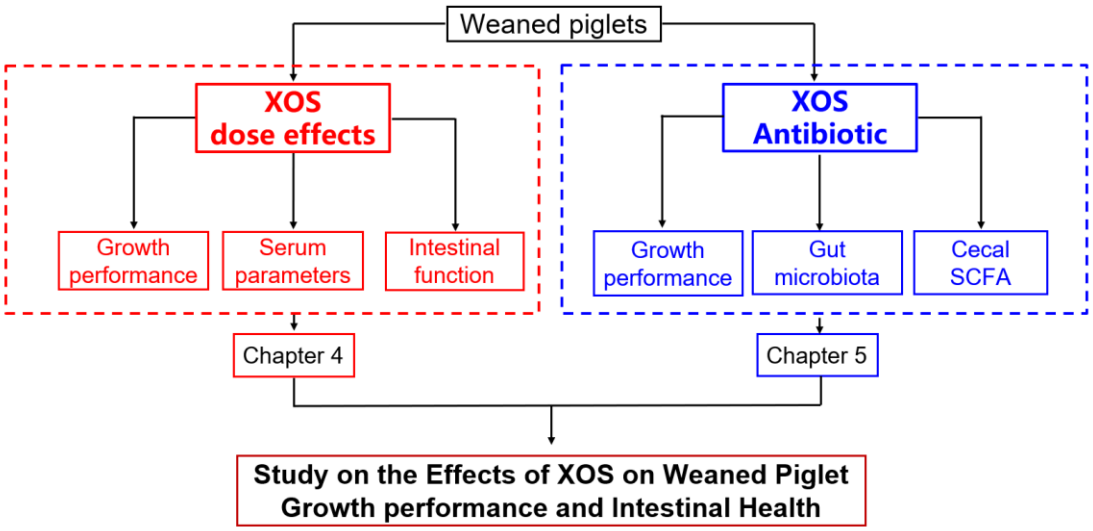


Figure 3-2: The technical route of the experiments performed during this thesis.

4

Effects of graded levels of xylo-oligosaccharides on growth performance, serum parameters, intestinal morphology and intestinal barrier function in weaned piglets

Background

Although XOS may be suggested to be a potential prebiotic for pigs, the dose to be administered in the post-weaning period remains to be determined. Therefore, the purpose of this experiment is to study the effect of different XOS doses on weaned piglets' performance, serum parameters, the intestinal morphology and the intestinal barrier in piglets.

Article 2

Effects of graded levels of xylo-oligosaccharides on growth performance, serum parameters, intestinal morphology and intestinal barrier function in weaned piglets

Yuxia Chen,[*†] Yining Xie,* Ruqing Zhong,* Hui Han,* Lei Liu,* Liang Chen, *[1]*

*Hongfu Zhang, *[2] Yves Beckers[†], and Nadia Everaert[†]*

*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China;

[†]Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, Liège University, Passage des Déportés 2, 5030 Gembloux, Belgium

[1] Corresponding author: chenliang01@caas.cn

The present chapter is adapted from: Effects of graded levels of xylo-oligosaccharides on growth performance, serum parameters, intestinal morphology, and intestinal barrier function in weaned piglets. (2021) *J. ANIM. SCI*. 99(7):skab183.

Abstract

The objective of this study was to investigate the effects of xylo-oligosaccharides (XOSs) supplementation on growth performance, serum parameters, small intestinal morphology, intestinal mucosal integrity, and immune function in weaned piglets. A total of 240 weaned piglets with an average body weight (BW) of 8.82 ± 0.05 kg (28 d of age) were assigned randomly to 4 dietary treatments in a 28-d trial, including a control diet (CON), 3 diets with XOSs supplementation at the concentration of 100, 500 and 1000 mg/kg (XOS100, XOS500, and XOS1000). There were 4 replicates per treatment with 15 pigs per pen. From d 1 to 14, there were no differences ($P > 0.05$) in average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) during the different treatments. The different XOS dose groups showed a quadratic effect on BW on day 28, ADG, and G:F day 1 to -28 of piglets ($P < 0.05$). From d 15 to 28, ADG of pigs fed the XOS500 diet was higher ($P < 0.05$) than pigs fed the CON diet. During the overall period (d 1 to 28), pigs fed the XOS500 diet had a higher BW, ADG and G:F than pigs fed the CON diet ($P < 0.05$). In addition, compared with the CON group, the XOS500 group had significantly higher serum total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD) and catalase (CAT) levels and lower malondialdehyde (MDA) levels on d 14 and 28 ($P < 0.05$). The serum immunoglobulin G (IgG) concentration in the XOS500 group was also significantly higher compared with the CON group on d 14 and 28 ($P <0.05$). However, serum immunoglobulin A (IgA) and immunoglobulin M (IgM) were not affected by the dietary treatments. Supplementation of XOS500 to the feed significantly increased the villus height (VH) and villus height to crypt depth ratio (VH:CD) in the jejunum and ileum in comparison with the CON and XOS1000 group. Moreover, the XOS500 group significantly elevated the expression levels of Occludin and zonula occludens protein-1 (ZO-1) in the ileum compared to the CON group. The ileal interleukin (IL)-1β, IL-8 and interferon (IFN)-γ mRNA expression levels in the XOS100 and XOS500 group were markedly lower than in the CON group. In contrast, the ileal IL-10 mRNA expression levels were remarkably higher in the XOS500 than CON group. In conclusion, xylo-oligosaccharides have a beneficial effect on growth performance by improving serum antioxidant defense system, serum IgG, small intestinal structure and intestinal barrier function in weaned piglets.

Key words: xylo-oligosaccharides, growth performance, serum parameters, small intestinal, weaned piglets

List of Abbreviations

XOSs, xylo-oligosaccharides; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; CAT, catalase; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; VH, villus height; CD, crypt depth; VH:CD, villus height to crypt depth; ZO-1, zonula occluden protein-1; IL-1β, interleukin-1β; IFN-γ, interferon-γ; AGP, antibiotic growth promoters; FCR, feed conversion ratio; qRT-PCR, quantitative real-time polymerase chain reaction;

1. Introduction

The commercial practice of weaning piglets induces different levels of stress due to a new environment and nutritional challenges, including the absence of sows, new penmates, and changes in the source and delivery of nutrients. Weaning may cause devastating characteristics including diarrhea, reducing feed efficiency, weight loss and in extreme cases death (Shin et al., 2019). To overcome the weaning stress, antibiotic growth promoters (AGP) were widely used since early 1950s at a sub-therapeutic dosage to improve health, optimize feed efficiency and promote animal growth in the swine industry (Cromwell, 2002). However, their potential side effects including the increased antibiotic resistance and residues in swine products have brought about a major concern for the modern society (Teillant, 2015). Therefore, there has been a considerable interest in using feed additives as an alternative to AGP in recent years. Different feed additives such as prebiotics, probiotics, organic acids, exogenous enzymes, and plant extracts have been investigated (Samal, 2009; Kiarie et al., 2013; Liu et al., 2018b).

Xylo-oligosaccharides (XOSs) are carbohydrate oligomers made up of 2-7 xylose units linked through β-(1→4)-linkages (Aachary and Prapulla, 2011; Samanta et al., 2015). XOS have been considered as a prebiotic. XOS have been found in some agricultural by-product, mainly including corncob, wheat bran, sugarcane residues and rice straw (Samanta et al., 2015). XOS have the highest stability at a pH range of 2.5–8 and exhibits high temperature resistance (Amorim et al., 2019). In 2015, XOS were evaluated by the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies requested by the European Commission, who concluded that XOS did not present any toxicity (Turck et al., 2018). Previous reports have demonstrated that XOS has the ability to achieve significant biological effects at low daily doses (Chen, et al., 2012; Suo, et al., 2015). Some studies have shown that 0.01-0.05% XOS supplementation can improve performance, intestinal characteristics, and egg quality of laying hens (Zhou, et al., 2009; Ding et al., 2018). In addition, some other biological benefits have been reported such as antioxidant activity (Yu et al., 2015), immunomodulatory and anti-inflammatory properties (Ding et al., 2018), or antimicrobial effects (Liu et al., 2018a; Chen et al., 2021). However, the effects of dietary XOS on growth performance, serum parameters or intestinal functions have not been fully studied in weaned piglets. The optimal dose of XOS used in the diet of weaned piglets is still unknown. Therefore, this study was to investigate the effects of dietary supplementation of different doses of XOS, with high purity, on growth performance, serum parameters, intestinal morphology, intestinal immune function and mucosal integrity.

2. Materials and methods

2.1. Animals, Diet and experimental Design

This study was approved by the Animal Welfare Committee of Institute of Animal Sciences, Chinese Academy of Agriculture Sciences (IASCAAS). All animal treatments in this study were performed according to the guidelines of the Animal Care and Use Committee of the Chinese Academy of Agriculture Sciences (CAAS). Animal care was practiced throughout the experiments and every effort was made to minimize suffering of piglets (Ethics Approval Code: IAS2019-34).

A total of 240 healthy weaned piglets (Duroc × Landrace × Large White, weaned at 28 d of age) with an average initial body weight (BW) of 8.82±0.05 kg, were randomly assigned to 4

treatments. The control group received a basal diet without any antibiotics or prebiotics (CON). The XOS treatment group received 100 (XOS100), 500 (XOS500) and 1000 (XOS1000) mg/kg corncob-derived XOS (Longlive Biotechnology Co. Ltd, Shandong, China) supplemented to the basal diet. This XOS has a purity of 95% XOS with a degree of polymerization (DP) 2-7 and is formed by xylose residues linked through β-(1,4)-linkages monomeric units. Prior to the trial, no clinical signs of diarrhea or other diseases were observed in the piglets. All pigs received similar husbandry practices. Each treatment had 4 replicate pens with 15 pigs per pen. All diets were manufactured in dry mash form and were formulated to meet or slightly exceed the nutritional requirements of the weaned pig as recommended by the NRC (2012, Table 1). The relative humidity and temperature of the piglet house were set at 60-65% and 25-28 °C, respectively. Piglets were allowed ad libitum access to feed and water throughout the experiment that lasted for 28 days.

Table 4-1. Ingredient and analyzed nutrient composition of basal diets.

Item	Basal diet
Ingredients, %	
Corn	59.00
Soybean meal	18.40
Fermented soybean meal	5.00
Fish meal	3.00
Soybean oil	2.50
Dried whey	5.00
Sugar	2.00
Glucose	2.00
Dicalcium phosphate	0.50
Limestone	0.50
Salt	0.30
Lysine HCl	0.40
Methionine	0.10
Threonine	0.10
Choline Chloride	0.10
Anti-mildew Agent	0.10
Premix[a]	1.00
Nutrient level	
Dry matter, %	87.80
Crude protein, %	20.00
Crude fiber, %	1.60
Neutral detergent fiber, %	22.90
Acid detergent fiber, %	3.70
Digestible energy, cal/g	3,502
Metabolizable energy, cal/g	3,243
Net energy, cal/g	2,553
Gross energy, cal/g	4,563

[a]The premix provided the following per kg of diet: vitamin A, 13,500 IU; vitamin D_3, 2,925 IU; vitamin E, 45 mg; vitamin K_3, 36.75 mg; vitamin B_1, 6.75 mg; vitamin B_2, 11.25 mg; vitamin B_6, 7.2mg; vitamin B_{12},0.054 mg; nicotinamide, 54 mg; calcium pantothenate, 15.75 mg; folic acid, 1.8mg; biotin, 0.342mg; Fe, 140mg; Cu, 20mg; Zn, 100mg; Mn, 30mg; I, 0.4mg; Se, 0.4mg.

2.2. Sample Collection and Measurements

Individual piglet BW was recorded initially, on d 14 and 28 of the experiment. and feed consumption per pen was recorded at the end of each phase (d 14 and 28) to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

On the morning of d 14 and 28, blood samples were collected from six piglets from each group via jugular vein puncture and 5 mL was collected into a vacutainer. After 2 h, the blood samples were centrifuged at 1600 × g at 4 °C for 15 min to recover serum, which was stored at - 20 °C until analysis.

On d 28, six piglets from each group were chosen randomly and euthanized aseptically. Afterwards, the entire intestine was removed from each pig. Segments of the ileum flushed with saline were collected for morphological examination. All intestinal segments were immediately fixed in 4% paraformaldehyde solution and then embedded in paraffin for intestinal morphology observation and mucosal samples were scraped using a scalpel blade and stored at −80 °C until further analysis.

2.3. Biochemical Analysis

Serum total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, malondialdehyde (MDA) and catalase (CAT) activity were measured by biochemical methods following the instructions of the corresponding reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The T-AOC was determined at 520 nm by the ferric reducing-antioxidant power assay. The activity of T-SOD was determined by the xanthine oxidase method using the T-SOD activity assay kit. The activity of GSH-Px was determined by using a GSH-Px kit. The MDA concentration was determined at 532 nm using the thiobarbituric acid method. The CAT activity was determined with CAT Assay Kit. The contents of serum immunoglobin A (IgA), immunoglobin G (IgG), and immunoglobin M (IgM) were measured by nephelometry (Beijing Kangjiahongyuan Biotechnology Institute, Beijing, P.R. China). Finally, these indices were calculated according to formulas in the assay kits.

2.4. Morphological Examination

Periodic Acid-Schiff (PAS) staining was performed according to standard protocols (Shatos et al., 2003). Paraformaldehyde-fixed duodenum, jejunum and ileum segments were dehydrated with ethanol, embedded in paraffin, and sectioned (5 μm). After dewaxing and immediately washing with distilled water for 1 min, the specimens were immersed in 0.5% periodate solution (Sigma Co.) for 5 min at room temperature in the dark. Afterwards, sections were immediately washed (30 s × 2) and soaked in Schiff's solution at 37°C. After 60 min, sections were washed twice with a sulfuric acid solution then quickly rinsed with distilled water. The subsequent steps followed the routine protocols of the laboratory. The sections were examined using light microscopy. The villus length and crypt depth were measured by random measurement of 10 villi and 10 measurements of the crypt per section using DS-U3 (Nikon, Japan).

2.5. Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from the ileal tissue samples with TB GreenTM Premix Ex TaqTM (Tli RNaseH Plus Reagent) (Takara Biotechnology, Dalian, China). Total RNA quantification and integrity were analyzed by adding 1 μL of each sample to a Bio-Rad CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). RNA was reverse-transcribed into complementary Deoxyribonucleic Acid (cDNA) with a Takara PrimeScriptTM RT Reagent Kit with gDNA Eraser (Takara Biotechnology, Dalian, China) according to the manufacturer's protocol. The synthesized cDNA was quantified and all tested samples were adjusted to the same concentration. The primer sequences for interleukin (IL)-1β, IL-6, IL-8, interferon (IFN)-γ, IL-10, zonula occludens protein-1 (ZO-1), occludin, claudin-2, and β-actin are shown in the Table 4-2. β-actin was the reference gene. Quantitative real-time RT-PCR (qRT-PCR) conditions were as follows: a single cycle of 30 sec at 95°C, followed by 40 cycles of 5 sec at 95°C, 30 sec at 60°C, and 15 sec at 72°C. Relative gene expression levels between the control and the various treatment groups were quantitated from the Ct (cycle threshold) value (Livak and Schmittgen, 2002).

Table 4-2. Primer sequences used for real-time PCR.

Items	Primer sequence (5' –3')
IL-1β	F: ACCTGGACCTTGGTTCTC
	R: GGATTCTTCATCGGCTTC
IL-6	F: GCATTCCCTCCTCTGGTC
	R: TCTTCAAGCCGTGTAGCC
IL-8	F: TACGCATTCCACACCTTTC
	R: GGCAGACCTCTTTTCCATT
IFN-γ	F: GGCCATTCAAAGGAGCATGG
	R: GCTCTCTGGCCTTGGAACAT
IL-10	F: TCGGCCCAGTGAAGAGTTTC
	R: GGAGTTCACGTGCTCCTTGA
ZO-1	F: CTCCAGGCCCTTACCTTTCG
	R: GGGGTAGGGGTCCTTCCTAT
Occludin	F: CAGGTGCACCCTCCAGATTG
	R: TATGTCGTTGCTGGGTGCAT
Claudin-2	F: GCATCATTTCCTCCCTGTT
	R: TCTTGGCTTTGGGTGGTT
β-actin	F: GCGTAGCATTTGCTGCATGA
	R: GCGTGTGTGTAACTAGGGGT

F means Forward and R means Reverse

2.6. Statistical analysis

The UNIVARIATE procedure was used to confirm the homogeneity of variance and also to analyze for outliers, but no outliers were identified. Data were analyzed using one-way ANOVA with Least Significant Difference (LSD) multiple comparison test. Linear and quadratic effects of the dietary XOS concentration were assessed by GLM procedure (SAS Version 9.2, SAS institute Inc., Cary, NC). The differences were considered significant if $P < 0.05$ and were

considered a trend if the P-value was between 0.05 and 0.10.

3. Results

3.1. Growth Performance

There was no difference in initial BW among the treatments (Table 4-3). The different XOS dose groups showed a quadratic effect on BW on day 28, ADG, and G:F day 1 to -28 of piglets ($P < 0.05$). Piglets in the XOS500 group had higher BW on d 28 than those in the CON and XOS1000 groups ($P < 0.05$). Piglets in the XOS500 group had higher ADG during d 1-28 than those in the CON or XOS1000 group ($P < 0.05$). Meanwhile, the XOS500 group had significantly better ADG and G:F in comparison with the CON group from d 1 to d 28 ($P < 0.05$). However, there were no differences in BW, ADG, ADFI or G:F between XOS100, XOS1000 and CON groups.

3.2. Antioxidant Defense System

In the present study, the effect of XOS on the antioxidant defense system was assessed by measuring the formation of MDA and the levels of key antioxidants as T-AOC, T-SOD, GSH-Px and CAT (Table 4-4). Compared with the CON group, the XOS500 group had higher T-SOD and CAT levels on d 14 and 28 ($P < 0.05$). In addition, the MDA level in the XOS500 group was lower than that of piglets in the CON group on d 14 and 28 ($P < 0.05$). No effect was observed for the XOS100 or XOS1000 groups compared to the CON group. The different XOS dose groups showed a quadratic effect on T-AOC, T-SOD, MDA and CAT levels on d 28 of piglets ($P < 0.05$).

3.3. Serum Immune Indices

As shown in Table 4-5, serum immunoglobulin G (IgG) concentrations on d 28 were higher in the XOS500 group compared with the CON group ($P <0.05$). However, no significant difference for IgA or IgM concentration was observed for the XOS100 or XOS1000 groups compared to the CON group. The different XOS dose groups showed a quadratic effect on IgG levels on d 28 of piglets ($P < 0.05$).

3.4. Intestinal Morphology

The effects of XOS on intestinal characteristics are shown in Table 4-6. There was no significant difference in villus height (VH), crypt depth (CD), or villus height to crypt depth (VH:CD) of the duodenum between treatments. The VH and VH:CD in both the jejunum and ileum were increased due to XOS500 supplementation compared to the CON or XOS1000 group ($P < 0.05$). In addition, the XOS100 treatment improved the VH:CD in the ileum compared to the CON group ($P < 0.05$). The different XOS dose groups showed a quadratic effect on the VH and VH:CD in both the jejunum and ileum on d 28 of piglets ($P < 0.05$).

3.5. Ileal Expression of Genes Related to Barrier Functions

The intestinal tight junction function was tested by analyzing ileal occludin, claudin-2 and ZO-1 expressions (Figure 4-1). Compared to the CON group, the XOS500 group had higher expression levels of occludin and ZO-1 in the ileum ($P < 0.05$). The expression of occludin and ZO-1 in the ileum of the XOS100 group was numerically higher than the CON group, but tend to be lower than the XOS500 group. However, dietary supplementation with XOS failed to alter the expression of claudin-2.

Table 4-3. Effect of graded levels of XOS (mg/kg feed) on growth performance of weaned piglets.

Item	CON	XOS100	XOS500	XOS1000	SEM	P-value	Linear	Quadratic
B W,[1] kg								
d 1	8.80	8.79	8.82	8.87	0.05	0.964	0.646	0.831
d 14	12.52	12.47	12.52	12.53	0.10	0.998	0.923	0.898
d 28	17.00^{b}	17.31^{ab}	17.80^{a}	17.24^{b}	0.09	0.024	0.115	0.017
ADG,[1] g/d								
d 1-14	266	263	264	262	4	0.993	0.835	0.971
d 15-28	321	346	377	336	9	0.209	0.351	0.090
d 1-28	293^{b}	304^{b}	321^{a}	299^{b}	3	0.012	0.150	0.006
ADFI,[1] g/d								
d 1-14	505	507	493	504	3	0.586	0.612	0.612
d 15-28	551	563	578	556	5	0.409	0.579	0.163
d 1-28	528	535	536	530	2	0.641	0.801	0.221
G:F[1]								
d 1-14	0.53	0.52	0.54	0.52	0.01	0.939	0.999	0.893
d 15-28	0.58	0.62	0.65	0.60	0.02	0.420	0.446	0.185
d 1-28	0.56^{a}	0.57^{ab}	0.60^{b}	0.56^{a}	0.01	0.033	0.247	0.019

CON = basal diet without XOS addition, XOS100 = 100mg/kg XOS addition dietary treatment, XOS500 = 500mg/kg XOS addition dietary treatment, XOS1000 = 1000mg/kg XOS addition dietary treatment.

$^{a, b}$ means in a row with different superscripts differ significantly ($P < 0.05$).

[1] BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed.

Table 4-4. Effect of graded levels of XOS (mg/kg feed) on serum antioxidant indices of weaned piglets.

Item	CON	XOS100	XOS500	XOS1000	SEM	P-value	Linear	Quadratic
d 14								
T-AOC[1], U/ml	4.5	4.7	5.1	4.8	0.08	0.174	0.136	0.199
T-SOD[2], U/ml	136.4^{b}	137.8^{b}	143.3^{a}	139.9^{ab}	0.76	0.018	0.025	0.125
GSH-Px[3], U/ml	701.8	721.7	735.4	711.7	5.21	0.211	0.398	0.065
MDA[4], nmol/ml	4.6^{a}	4.5^{ab}	4.1^{b}	4.5^{ab}	0.08	0.138	0.354	0.092
CAT[5], U/ml	3.3^{b}	3.9^{ab}	4.2^{a}	3.5^{b}	0.10	0.024	0.338	0.005
d 28								
T-AOC, U/ml	4.3^{b}	5.2^{ab}	5.6^{a}	5.0^{ab}	0.14	0.031	0.065	0.017
T-SOD, U/ml	137.1^{b}	142.3^{ab}	144.9^{a}	139.6^{ab}	0.94	0.038	0.229	0.010
GSH-Px, U/ml	732.2	759.4	750.6	736.4	8.27	0.735	0.964	0.295
MDA, nmol/ml	4.5^{a}	4.0^{ab}	3.6^{b}	4.2^{a}	0.08	0.007	0.116	0.003
CAT, U/ml	3.3^{b}	4.2^{a}	4.5^{a}	4.3^{a}	0.11	<0.001	<0.001	0.002

CON = basal diet without XOS addition, XOS100 = 100mg/kg XOS addition dietary treatment, XOS500 = 500mg/kg XOS addition dietary treatment, XOS1000 = 1000mg/kg XOS addition dietary treatment.

$^{a, b}$ means in a row with different superscripts differ significantly ($P < 0.05$).

[1]T-AOC, total antioxidant capacity.

[2]T-SOD, total superoxide dismutase.

[3]GSH-Px, glutathione peroxidase.

[4]MDA, malondialdehyde.

[5]CAT, catalase.

Table 4-5. Effect of graded levels of XOS (mg/kg feed) on serum immunoglobulins of weaned piglets.

Item	CON	XOS100	XOS500	XOS1000	SEM	P-value	Linear	Quadratic
d 14								
IgA[1], mg/ml	1.03	1.10	1.19	1.14	0.02	0.149	0.058	0.241
IgG[2], mg/ml	7.40	7.87	8.23	7.95	0.10	0.068	0.043	0.088
IgM[3], mg/ml	0.91	0.91	0.99	0.99	0.02	0.300	0.085	0.962
d 28								
IgA, mg/ml	1.02	1.06	1.03	1.05	0.01	0.833	0.732	0.799
IgG, mg/ml	7.19^{c}	7.97^{ab}	8.20^{a}	7.58^{bc}	0.10	0.002	0.072	<0.001
IgM, mg/ml	0.98	0.91	0.96	0.98	0.02	0.587	0.756	0.281

CON = basal diet without XOS addition, XOS100 = 100mg/kg XOS addition dietary treatment, XOS500 = 500mg/kg XOS addition dietary treatment, XOS1000 = 1000mg/kg XOS addition dietary treatment.

$^{a\text{-}c}$ means in a row with different superscripts differ significantly ($P < 0.05$).

[1]IgA, immunoglobulin A;

[2]IgG, immunoglobulin G;

[3]IgM, immunoglobulin M.

Table 4-6. Effect of graded levels of XOS (mg/kg feed) on intestinal morphology of weaned piglets.

Item	CON	XOS100	XOS500	XOS1000	SEM	P-value	Linear	Quadratic
Duodenum								
VH[1], μm	500.1	507.5	510.8	505.9	2.94	0.728	0.499	0.371
CD[2], μm	230.5	222.2	209.2	223.5	3.46	0.274	0.325	0.149
VH:CD[3]	2.21	2.31	2.39	2.22	0.03	0.282	0.767	0.079
Jejunum								
VH, μm	421.0^{b}	430.6^{ab}	442.5^{a}	425.5^{b}	2.31	0.014	0.226	0.007
CD, μm	196.9	194.9	192.9	195.5	1.27	0.815	0.642	0.442
VH:CD	2.14^{b}	2.25^{ab}	2.30^{a}	2.18^{b}	0.02	0.029	0.375	0.005
Ileum								
VH, μm	377.0^{c}	400.2^{ab}	411.8^{a}	387.3^{bc}	3.41	0.004	0.152	<0.001
CD, μm	130.2	128.6	127.4	127.6	0.99	0.806	0.371	0.702
VH:CD	2.90^{b}	3.11^{a}	3.23^{a}	3.04^{ab}	0.03	0.001	0.001	<0.001

CON = basal diet without XOS addition, XOS100 = 100mg/kg XOS addition dietary treatment, XOS500 = 500mg/kg XOS addition dietary treatment, XOS1000 = 1000mg/kg XOS addition dietary treatment.

$^{a\text{-}c}$ means in a row with different superscripts differ significantly ($P < 0.05$).

[1]VH, villus height.

[2]CD, crypt depth.

[3]VH:CD, villus height to crypt depth.

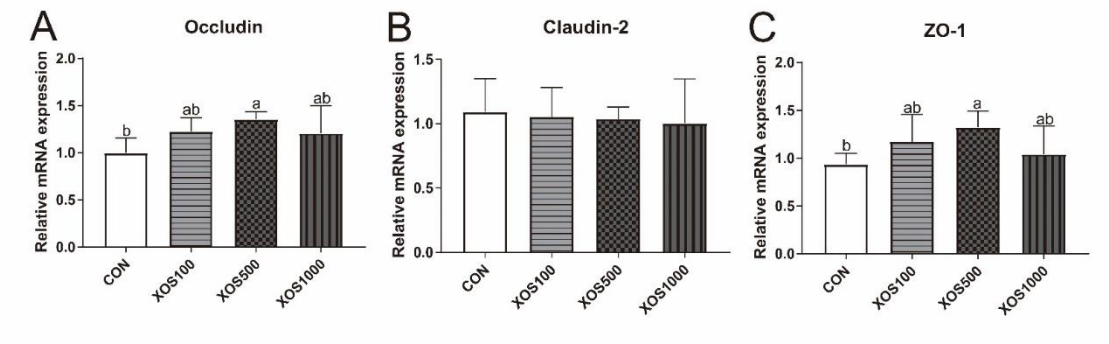


Figure 4-1. The relative mRNA expression of intestinal epithelium integrity-related genes in the ileal tissues of piglets. (A)Occludin (B)Claudin-2 (C)ZO-1. Groups with no superscript letter or the same superscript letter are not significantly different ($P > 0.05$); those with different superscript letters are significantly different ($P < 0.05$). CON = basal diet without XOS addition, XOS100 = 100mg/kg XOS addition dietary treatment, XOS500 = 500mg/kg XOS addition dietary treatment, XOS1000 = 1000mg/kg XOS addition dietary treatment.

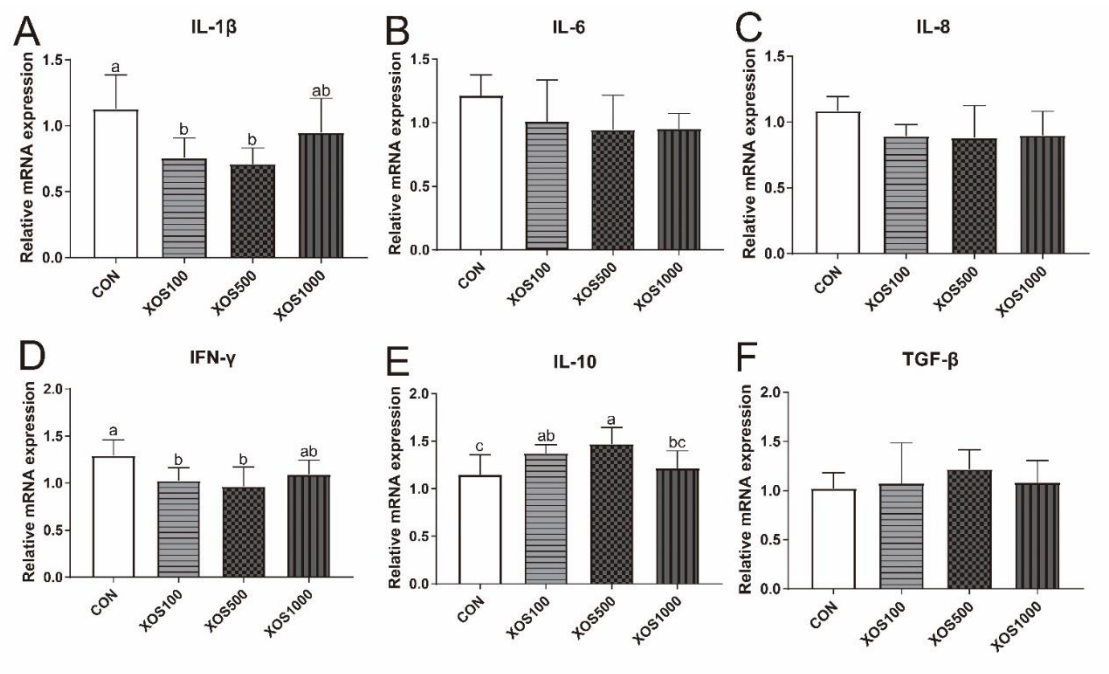


Figure 4-2. The relative mRNA expression of intestinal epithelium immune-related genes in the ileal tissues of piglets. (A) IL-1β (B)IL-6 (C)IL-8 (D)IFN-γ (E) IL-10 (F)TGF-β. Groups with no superscript letter or the same superscript letter are not significantly different ($P > 0.05$); those with different superscript letters are significantly different ($P < 0.05$). CON = basal diet without XOS addition, XOS100 = 100mg/kg XOS addition dietary treatment, XOS500 = 500mg/kg XOS addition dietary treatment, XOS1000 = 1000mg/kg XOS addition dietary treatment.

3.6. Ileal Expression of Genes Related to the Inflammatory Response

The inflammatory cytokine mRNA expression levels are shown in Figure 4-2. The ileal IL-1β and IFN-γ mRNA expression levels in the XOS100 or XOS500 group were lower than the CON group ($P < 0.05$). In contrast, the ileal IL-10 mRNA expression levels were higher in the XOS500 than the CON or XOS1000 group ($P < 0.05$).

4. Discussion

Xylo-oligosaccharides are considered as promising prebiotics. In our study, XOS500 significantly increased BW, ADG and ADFI and decreased G:F of piglets, which was consistent with previous studies. For example, Liu et al. reported that 200 mg/kg XOS with a purity of 50% remarkably increased ADG and feed efficiency in weaned piglets (Liu et al., 2018a). Moreover, several studies showed positive dose effects of XOS on growth performance in broilers. Pourabedin et al. demonstrated that feed conversion ratio (FCR) in broilers fed 2 g/kg XOS diets was significantly lower than those fed a control diet or 1 g/kg XOS between days 7 and 21 (Pourabedin et al., 2015). In contrast, Yin et al. found that 0.01% XOS with a purity of 40% had no significant improvement in the growth performance of the piglets (Yin et al., 2019), which might mainly be explained by the low dose. The different XOS dose groups showed a quadratic effect on BW on day 28, ADG, and G:F day 1 to -28 of piglets. The XOS1000 in our study significantly failed to improve the growth performance in weaned piglets, which may be due to an excessive dose of XOS. Therefore, we speculate the XOS500 supplementation is optimal dose during these 3 dose groups. Other oligosaccharides, for example, chitooligosaccharides have also been shown to have a dose-effect affecting the growth performance in pigs (Liu et al., 2010). These results on the growth performance indicate that there will be an optimal XOS dose for weaned piglets.

Serum biochemical parameters are often used to evaluate the physiological effects of nutrients in animals. Antioxidant parameters are regarded as important serum indices to assess animals' health. Changes in the antioxidant defense system, mainly including T-AOC, T-SOD, CAT and GSH-Px, may indicate oxidative stress (Zhu et al., 2012). The generation and elimination of free radicals are in a dynamic balance and can prevent diseases by maintaining a favorable and harmless level (Lobo et al., 2010). Serum T-AOC could scavenge free radicals from a specific organ or living organism, and its concentration reflects the total antioxidant ability (Wang et al., 2008). T-SOD is a well-known endogenous protective enzyme that acts as a component of the first line defense system against reactive oxygen species (ROS). It breaks down hydrogen peroxides and hydroperoxides into less toxic molecules (H_2O_2/alcohol and O_2) (Ighodaro and Akinloye, 2017). CAT reduces H_2O_2 to O_2 and H_2O, consequently finishing the detoxification process (Chelikani et al., 2004). GSH-Px is also an important antioxidant enzyme that converts hydrogen peroxides to water (Komatsu et al., 2003). Circulating MDA is one of the common and widely used biomarkers of oxidative stress. MDA is the most familiar degradation product of lipid peroxidation and could result in cell injury including cell senescence and even apoptosis (Han, 2018). In the present study, the results showed that increases of T-AOC, T-SOD and CAT activities were found in the piglets supplemented with XOS500 on d 14 and 28 compared to the CON group. In addition, the MDA level in the XOS500 group was significantly lower than that of piglets in the CON group on d 14 and 28. These results indicate that the antioxidant capacity of piglets was improved with XOS500 supplementation, while, as for the performance parameter, there seems to be an optimal dose as neither the XOS100 nor the XOS1000 obtained this positive effect on the antioxidant status. Consistent with these findings, a previous study also revealed that wheat bran xylooligosaccharides could increase antioxidant status in rats fed a high-fat diet (Wang et al., 2011). Additionally, the research of Japtap revealed that a XOS mixture also exhibited concentration-dependent antioxidant activity (Jagtap et al., 2017). The serum immunoglobins—

IgG, IgA, and IgM could protect the extravascular compartment against pathogenic viruses and microorganisms (Li et al., 2007). In this study, the results showed that serum IgG concentration on d 14 and 28 were significantly higher in the XOS500 group compared with the CON group. These results indicate that XOS may play a very important role in improving the immune function of piglets. Similarly, Abdelmakek et al. also reported that XOS treatment significantly increased the serum immunoglobulin compared with the control in Dicentrarchus labrax fingerlings (Abdelmalek et al., 2015). The increase in antioxidant capacity by XOS supplementation might be responsible for the observed effects on the improvement of the immune function. As mentioned above, the underlying mechanisms for the effects of XOS are likely to be related to changes in the intestinal and systemic immune network. But further research is necessary to clarify the complete working mechanisms.

The intestinal morphology indices such as VH, CD and VH:CD ratio are often used as a criterion to estimate the nutrient digestion and absorption capacity of the small intestine. In this study, we found that the VH and VH:CD ratio of jejunum and ileum significantly increased in the XOS500 fed pigs compared to the CON group. However, XOS100 and XOS1000 supplementation group could not significantly improve the intestinal structure. These results indicate that the optimal dose of XOS may protect the intestine against villous atrophy and epithelial cell necrosis. These results are in line with previous studies, that demonstrated that using 200 mg/kg XOS with a purity of 50% not only improved the VH:CD ratio of the jejunum but also increased the apparent total tract digestibility of dry matter and gross energy on d14 in the piglets (Liu et al., 2018a). Maesschalck et al. also confirmed that supplementation of 0.5% XOS with a purity of 35% to the broiler feed significantly increased the villus height in the ileum (Maesschalck et al., 2015). Similarly, Ding et al. demonstrated that there was a linear improvement in villus height and VH: CD ratio of the jejunum as dietary XOS concentration increased in laying hens (Ding et al., 2018). However, the addition of 0.01% XOS with a purity of 40% in the diet of weaned piglets had little effects on the intestinal structure and villus surface area (Yin et al., 2019). In addition, Suo et al. found that the addition of XOS with a purity of 35% in the diet decreased the crypt depth of the duodenum in broilers (Suo, 2015; Yin et al., 2019). These results suggest that an appropriate dose of XOS can be a promising approach for maintaining the intestinal epithelium in piglets. Hence, a possible explanation for the improvement of growth performance is that XOS500 supplementation improved intestinal morphology and gut absorptive function.

An intact intestinal barrier plays a crucial role in preventing luminal harmful molecules, such as pathogens, toxins and antigens, from penetrating the mucosa (Martin-Venegas and R., 2006). Tight junctions are the crucial components of the intestinal mucosal barrier and exert a pivotal role in the maintenance of the barrier function. They are multiple protein complexes consisting of the transmembrane proteins *occludin* and *claudin-2* and the cytosolic protein *ZO-1*. We found that XOS500 upregulated *occludin* and *ZO-1* mRNA levels in the ileal mucosa in weaned pigs, indicating that dietary supplementation with XOS enhances the intestinal barrier integrity in weaned pigs. In a recent paper, Yin et al. have shown that XOS supplementation could markedly enhance *ZO-1* expression in the piglets (Yin et al., 2019). Gut barrier function is also tightly associated with inflammation responses. Pro-inflammatory cytokines such as *IL-1β*, *IL-6*, *IL-8* and *IFN-γ* and anti-inflammatory cytokines including *IL-10* and *Transforming Growth Factor-β* (*TGF-β*)

are essential for mediating inflammatory responses. Thus, we further determined ileal inflammatory cytokines and the results demonstrated that XOS100 and XOS500 treatments remarkably reduced the *IL-1β* and *IFN-γ* mRNA expression. Additionally, XOS500 supplementation significantly upregulated *IL-10* mRNA levels. These results indicate that XOS500 supplementation may improve the inflammatory status in piglets. Similarly, Hansen et al. found that xylo-oligosaccharides could downregulate *IFN-γ* and low-grade inflammatory cytokine *IL-1β* in mice (Hansen et al., 2013). Yuan et al. also revealed that XOS supplementation reduced mRNA expression of *IFN-γ* in broilers (Yuan et al., 2018). Therefore, dietary supplementation with XOS contributes to improve the inflammatory status. The improvement of intestinal barrier integrity and inflammatory status might be correlated with the improved intestinal morphology.

In conclusion, the results of the present study demonstrated that XOS500 supplementation could effectively increase the growth performance through enhancing the serum antioxidant defense system, elevating the serum IgG, improving the small intestinal structure, and maintaining intestinal barrier function in weaned piglets. Further studies should be done to fine-tune the optimal dose.

5. References

Aachary, A. A., and S. G. Prapulla. 2011. Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. Compr Rev Food Sci F 10(1):2-16. doi:10.1111/j.1541-4337.2010.00135.x.

Abdelmalek, B. E., D. Driss, F. Kallel, M. Guargouri, H. Missaoui, S. E. Chaabouni, M. A. Ayadi, and A. Bougatef. 2015. Effect of xylan oligosaccharides generated from corncobs on food acceptability, growth performance, haematology and immunological parameters of Dicentrarchus labrax fingerlings. Fish Physiol Biochem 41(6):1587-1596. doi: 10.1007/s10695-015-0110-5.

Amorim, C., S. C. Silvério, K. L. J. Prather, and L. R. Rodrigues. 2019. From lignocellulosic residues to market: Production and commercial potential of xylooligosaccharides. Biotechnol Adv 37(7):107397-. doi: 10.1016/j.biotechadv.2019.05.003.

Chelikani, P., I. Fita, and P. C. Loewen. 2004. Diversity of structures and properties among catalases. Cell Mol Life Sci 61(2):192-208. doi: 10.1007/s00018-003-3206-5.

Chen H. H., Y. K. Chen, H. C. Chang, Y. L. Su. 2012. Immunomodulatory Effects of Xylooligosaccharides[J]. Food Sci Technol Res 18(2):195-199. doi: 10.3136/fstr.18.195.

Chen Y. X., Y. N. Xie, R. Q. Zhong, L. Liu, C. G. Lin, L. Xiao, L. Chen, H. F. Zhang, B. Yves and E. Nadia. 2021. Effects of Xylo-Oligosaccharides on Growth and Gut Microbiota as Potential Replacements for Antibiotic in Weaning Piglets. Front. Microbiol. 12(2021). doi: 10.3389/fmicb.2021.641172.

Cromwell, G. L. 2002. Why and how antibiotics are used in swine production. Anim Biotechnol 13(1):7-27. doi: 10.1081/abio-120005767.

De Maesschalck, C., Eeckhaut, V., Maertens, L., De Lange, L., Marchal, L., C., Nezer, et al. 2015. Effects of xylo-oligosaccharides on broiler chicken performance and microbiota. Appl. Environ. Microbiol. 81(17): 5880-5888. doi: 10.1128/AEM.01616-15.

Ding, X. M., D. D. Li, S. P. Bai, J. P. Wang, Q. F. Zeng, Z. W. Su, Y. Xuan, and K. Y. Zhang. 2018. Effect of dietary xylooligosaccharides on intestinal characteristics, gut microbiota, cecal short-chain fatty acids, and plasma immune parameters of laying hens. Poultry Sci 97(3):874-881. doi: 10.3382/ps/pex372.

Eleventh, R., Ed.;National Research Council. Nutrient Requirements of Swine[J]; National Academies Press: Washington, DC, USA, 2012; ISBN 978-0-309-22423-9.

Han, Y. S., C. H. Tang , Q. Y. Zhao; T. F. Zhan, K. Zhang, Y. M. Han, J. M. Zhang. 2018. Effects of dietary supplementation with combinations of organic and medium chain fatty acids as replacements for chlortetracycline on growth performance, serum immunity, and fecal microbiota of weaned piglets. Livest Sci 216:210-218. doi: 10.1016/j.livsci.2018.08.013.

Hansen, C. H. F., H. Frokiaer, A. G. Christensen, A. Bergstrom, T. R. Licht, A. K. Hansen, and

S. B. Metzdorff. 2013. Dietary Xylooligosaccharide Downregulates IFN-[Gamma] and the Low-Grade Inflammatory Cytokine IL-1[beta] Systemically in Mice1-3. J Nutr 143(4):533-540. doi: 10.3945/jn.112.172361.

Lghodaro, O. M., and O. A. Akinloye. 2017. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex J Med:S2090506817301550. doi: 10.1016/j.ajme.2017.09.001.

Jagtap, S., R. A. Deshmukh, S. Menon, and S. Das. 2017. Xylooligosaccharides production by crude microbial enzymes from agricultural waste without prior treatment and their potential application as nutraceuticals. Bioresour Technol 245(Pt A):283-288. doi: 10.1016/j.biortech.2017.08.174.

Kiarie, E., L. F. Romero, and C. M. Nyachoti. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutr Res Rev 26(01):71-88. doi: 10.1017/S0954422413000048.

Komatsu, W., K. Ishihara, M. Murata, H. Saito, and K. Shinohara. 2003. Docosahexaenoic acid suppresses nitric oxide production and inducible nitric oxide synthase expression in interferon-gamma plus lipopolysaccharide-stimulated murine macrophages by inhibiting the oxidative stress. Free Radic Biol Med 34(8):1006-1016. doi: 10.1016/s0891-5849(03)00027-3

Li, P., Y. L. Yin, D. Li, S. W. Kim, and G. Wu. 2007. Amino acids and immune function. Br J Nutr 98(2):237-252. doi: 10.1017/s000711450769936x.

Liu, J., S. Cao, J. Liu, Y. Xie, and H. Zhang. 2018a. Effect of probiotics and xylo-oligosaccharide supplementation on nutrient digestibility, intestinal health and noxious gas emission in weanling pigs. Asian-Australas J Anim Sci 31(10):1660-1669. doi: 10.5713/ajas.17.0908.

Liu, P., X. S. Piao, P. A. Thacker, Z. K. Zeng, & S. W. Kim. 2010. Chito-oligosaccharide reduces diarrhea incidence and attenuates the immune response of weaned pigs challenged with escherichia coli k88. J Anim Sci., 88(12), 3871-3879. doi: 10.2527/jas.2009-2771.

Liu, Y., C. D. Espinosa, J. J. Abelilla, G. A. Casas, L. V. Lagos, S. A. Lee, W. B. Kwon, J. K. Mathai, D. Navarro, and N. W. Jaworski. 2018b. Non-antibiotic feed additives in diets for pigs:A review. Anim Nutr. 4(2):113-125. doi: 10.1016/j.aninu.2018.01.007.

Livak, K. J., and T. D. Schmittgen. 2002. Analysis of Relative Gene Expression Data using Real-Time Quantitative PCR. Methods. 25(4):402-8. doi: 10.1006/meth.2001.1262.

Lobo, V., A. Patil, A. Phatak, and N. Chandra. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 4(8):118-26. doi: 10.4103/0973-7847.70902.

Martin-Venegas, R., S. Roig-Perez, R.Ferrer, and J. Moreno. 2006. Arachidonic acid cascade and epithelial barrier function during Caco-2 cell differentiation. J Lipid Res. 47(7):1416-23. doi: 10.1194/jlr.M500564-JLR200.

Pourabedin, M., L. Guan, and X. Zhao. 2015. Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. Microbiome. 10;3:15. doi: 10.1186/s40168-015-0079-4. eCollection 2015.

Samal, L., and C. Behura 2009. Prebiotics: An Emerging Nutritional Approach for Improving Gut Health of Livestock and Poultry. Asian J Anim Vet Adv, 10(11), 724-739. doi: 10.3923/ajava.2015.724.739.

Samanta, A. K., N. Jayapal, C. Jayaram, S. Roy, A. P. Kolte, S. Senani, and M. Sridhar. 2015. Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. Curr Protein Pept Sci. 19(1):48-67. doi: 10.2174/1389203717666160923155209.

Shatos, M. A., J. D. Ríos, Y. Horikawa, R. R. Hodges, E. L. Chang, C. R. Bernardino, P. A. Rubin, and D. A. Dartt. 2003. Isolation and characterization of cultured human conjunctival goblet cells. Invest Ophthalmol Vis Sci 44(6):2477-2486. doi: 10.1167/iovs.02-0550

Shin, D., S. Y. Chang, P. Bogere, K. Won, J. Y. Choi, Y. J. Choi, H. K. Lee, J. Hur, B. Y. Park, Y. Kim, and J. Heo. 2019. Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. PloS one 14(8):e0220843. doi: 10.1371/journal.pone.0220843

Suo, H. Q., L. Lin, G. H. Xu, L. Xiao, X. G. Chen, R. R. Xia, L. Y. Zhang, X. G. Luo. 2015. Effectiveness of dietary xylo-oligosaccharides for broilers fed a conventional corn-soybean

meal diet. J Integr Agr, 14(010):2050-2057. doi: 10.1016/S2095-3119(15)61101-7.

Teillant, A., C. Brower, and R. Laxminarayan. 2015. Economics of Antibiotic Growth Promoters in Livestock. Annu Rev Resour Econ, 7(1), 349–374. doi: 10.1146/annurev-resource-100814-125015.

Turck, D., J. L. Bresson, B. Burlingame, T. Dean, S. Fairweather-Tait, M. Heinonen, K. I. Hirsch-Ernst, I. Mangelsdorf, H. J. Mcardle, and A. Naska, et al. 2018. Safety of xylooligosaccharides (XOS) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 16(7). doi: 10.2903/j.efsa.2018.5361.

Wang, J., Y. Cao, C. Wang, and B. Sun. 2011. Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet. Carbohyd Polym 86(3):1192-1197. doi: 10.1016/j.carbpol.2011.06.014.

Wang, Y. Z., C. L. Xu, Z. H. An, J. X. Liu, and J. Feng. 2008. Effect of dietary bovine lactoferrin on performance and antioxidant status of piglets. Anim Feed Sci Tech 140(3-4):326-336. doi: 10.1016/j.anifeedsci.2007.02.006.

Yin, J., F. Li, X. Kong, C. Wen, Q. Guo, L. Zhang, W. Wang, Y. Duan, T. Li, Z. Tan, and Y. Yin. 2019. Dietary xylo-oligosaccharide improves intestinal functions in weaned piglets. Food Funct 10(5):2701-2709. doi: 10.1039/c8fo02485e.

Yu, X., J. Yin, L. Li, C. Luan, and S. Li. 2015. Prebiotic Potential of Xylooligosaccharides Derived from Corn Cobs and Their In Vitro Antioxidant Activity When Combined with Lactobacillus. J Microbiol Biotechnol . 25(7):1084-92. doi: 10.4014/jmb.1501.01022.

Yuan, L., W. Li, Q. Huo, C. Du, Z. Wang, B. Yi, and M. Wang. 2018. Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens. PeerJ. 5;6:e4435. doi: 10.7717/peerj.4435. eCollection 2018.

Zhou, E., X. Pan, and X. Tian. 2009. Application Study of Xylo-oligosaccharide in Layer Production. Modern Applied ence, 3(1):103-107. doi: 10.5539/mas.v3n1p103.

Zhu, L. H., K. L. Zhao, X. L. Chen, and J. X. Xu. 2012. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. J Anim Sci. 90(8):2581-9. doi: 10.2527/jas.2012-4444.

5

Effects of Xylo-oligosaccharides on Growth and Gut Microbiota as Potential Replacements for Antibiotic in Weaning Piglets

Background

In the previous chapter, the best dose, positively affecting growth performance, was 500 mg XOS/kg diet. In this chapter, we investigated if this XOS dose could be considered as a potential replacement for antibiotic, optimizing the gut morphology, gut microbial and metabolic composition of weaned piglets. We therefore compared the XOS dose with a positive control containing 100 mg/kg chlortetracycline, and a negative control group (non-supplemented).

Article 3

Effects of Xylo-oligosaccharides on Growth and Gut Microbiota as Potential Replacements for Antibiotic in Weaning Piglets

Yuxia Chen [1,2†], Yining Xie [1,3†], Ruqing Zhong[1], Lei Liu[1], Changguang Lin[4], Lin Xiao[5], Liang Chen[1], Hongfu Zhang[1 *], Yves Beckers[2], Nadia Everaert[2]*

[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China; yuxiatianya0428@126.com

[2]Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, Liège University, Passage des Déportés 2, Gembloux, Belgium

[3]School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, China

[4]Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agriculture Sciences, Fuzhou 350003, China

[5]Shandong Longlive Bio-technology Co., Ltd., Yucheng 251200, China

*Correspondence:

chenliang01@caas.cn, zhanghongfu@caas.cn; Tel.: +86-10-6281-8910

†These authors have contributed equally to this work

This chapter was adapted from the manuscript published in Frontiers in Microbiology. (2021), 12:641172. doi: 10.3389/fmicb.2021.641172.

Abstract

Xylo-oligosaccharides (XOS) is a well-known kind of oligosaccharide and extensively applied as a prebiotic. The objective of this study was to investigate the effect of XOS supplementation substituting chlortetracycline on growth, gut morphology, gut microbiota, and hindgut short chain fatty acid contents of weaning piglets. A total of 180 weaned piglets were randomly allocated to three treatments for 28 days, as follows: control group (basal diet, CON), basal diet with 500mg/kg (XOS500) XOS, and positive control (basal diet with 100 mg/kg chlortetracycline, CTC). Compared with the CON group, the piglets in the XOS500 group improved body weight on days 28, average daily gain and reduced feed : gain ratio during days 1–28 ($P < 0.05$). The XOS500 supplementation increased Villus Height and Villus Height : Crypt Depth Ratio in the ileum ($P < 0.05$). Villus Height : Crypt Depth of the ileum was also increased in the CTC treatment group ($P < 0.05$). Meanwhile, the XOS500 supplementation increased significantly the numbers of goblet cells in the crypt of the cecum. High-throughput 16S rRNA gene sequencing revealed distinct differences in microbial compositions between the ileum and cecum. XOS500 supplementation significantly increased the bacterial diversity. However, CTC treatment markedly reduced the microbial diversity ($P < 0.05$). Meanwhile, XOS500 supplementation in the diet significantly increased the abundance of *Lactobacillus* genus compared to the CON and CTC group in the ileum and cecum ($P < 0.01$), whereas the level of *Clostridium_sensu_stricto_1*, *Escherichia-Shigella* and *Terrisporobacter* genus in the XOS500 group were markedly lower than the CON and CTC group ($P < 0.05$). In addition, dietary supplementation with XOS500 significantly increased the total short-chain fatty acids, propionate and butyrate concentrations and decreased the acetate concentration compared to the CON group in the cecum ($P < 0.05$). In summary, dietary supplemented with XOS500 could enhance specific beneficial microbiota abundance and decrease harmful microbiota abundance to maintain the structure of the intestinal morphology and improve growth performance of weaned piglets. Thus, XOS may potentially function as an alternative to in-feed antibiotics in weaned piglets in modern husbandry.

Keywords: xylo-oligosaccharides, growth performance, gut microbiota, antibiotics, weaned piglets

1. Introduction

Weaning piglets in modern swine industry are often challenged by post-weaning stresses including dietary, social, and environmental changes. These stresses result in increasing disease and mortality risks, reducing growth rates and rising impairment of the intestinal microbiota. Every year, 17% of piglets born in Europe died during weaning due to opportunistic pathogen infection (Gresse et al. 2017). China is the biggest pork producer and consumer in the world but about 24 million weaning piglets every year die from diarrhea due to inappropriate treatment during weaning (Wang et al. 2019).

Antibiotics were widely used in weanling piglets to promote animal growth and prevent infections (Cromwell 2002; Wijtten et al. 2011; Hu et al. 2020). However, the addition of antibiotics in feed can result in changes in the intestinal microbiota due to its broad-spectrum antibacterial activity (Neuman et al. 2018). In addition, antibiotics resistance and antimicrobial residues has become a major threat in treating pathogenic bacterial infections (Toutain et al. 2016). For example, apramycin sulfate was widely used in China to prevent piglet diarrhea, however, taking apramycin might cause cross-resistance of apramycin/gentamicin in Escherichia coli and S. Enteritidis (Herrero-Fresno et al. 2016). For these reasons, the European Commission decided to ban the use of antibiotics as feed additives since January 2006 due to the risk of spreading antibiotic resistance (Smith et al. 2010). Other countries are also trying to gradually reduce or forbid use of feed antibiotics. For instance, the use of colistin sulphate as feed additives in animal diets has been banned in China since April, 2017 and India since July, 2019 (Wang et al. 2020). Therefore, it is urgent to develop novel alternatives to antibiotic feed additives.

Recently, several alternatives to antibiotics were reported to maintain swine health and improve growth performance, including probiotics, prebiotics, acidifiers, and essential oils (Gresse et al. 2017; Wang et al. 2019). Prebiotics are defined as a substrate that is selectively utilized by host microorganisms conferring benefits upon host health (Gibson et al. 2017). Collaway and colleagues showed that prebiotics are a preferable alternative to antibiotics (Callaway et al. 2008). Commercially available prebiotics mainly include xylo-oligosaccharides (XOS), fructo-oligosaccharides (Mikkelsen et al. 2003), inulin (Mair et al. 2010), mannan-oligosaccharides (Zhao et al. 2012), galacto-oligosaccharides (Alizadeh et al. 2015), and transgalacto-oligosaccharides (Mikkelsen and Jensen 2004). XOS are sugar oligomers made up of 2-6 xylose units linked through β-(1→4)-linkages (Samanta et al. 2015). XOS have been demonstrated to improve animal health, growth performance, and enhance the role of endogenous beneficial microbiota, such as bifidobacterium and lactic acid bacteria in the gut (Gobinath et al. 2010; De Maesschalck et al. 2015; Yang et al. 2015). However, the effect of XOS as antibiotic substitution in weaned piglets has not been reported until now. Therefore, this study aimed to investigate the effects of dietary supplementation with XOS as potential replacements for antibiotic on the growth, gut morphology, gut microbiota, and hindgut SCFA contents of weaned piglets.

2. Materials and methods

2.1. Animals and Experimental Design

This study was approved by the Animal Welfare Committee of Institute of Animal Sciences, Chinese Academy of Agriculture Sciences (IASCAAS). All animal treatments in this study

were performed according to the guidelines of the Animal Care and Use Committee of the Chinese Academy of Agriculture Sciences (CAAS). Humane animal care was practiced throughout the experiments and every effort was made to minimize suffering for piglets (Ethics Approval Code: IAS2019-34).

A total of 180 healthy weaned piglets (Duroc × Landrace × Large White, weaned at 28 d of age) with an average initial body weight (BW) of 8.84±0.25 kg, were randomly assigned to 3 treatments based on the BW and sex. The control group with basal diet without any antibiotics or prebiotics (CON), and the antibiotic group with basal diet supplemented with 100 mg/kg pure chlortetracycline (CTC) were attributed as the positive control group. The XOS treated group piglets were fed 500 (XOS500) mg/kg corncob-derived XOS (Longlive Biotechnology Co. Ltd, Shandong, China). This XOS have a purity of 95% a degree of polymerization (DP) 2-7 and is formed by xylose residues linked through β-(1,4)-linkages monomeric units. Prior to the trial, no clinical signs of diarrhea or other diseases were observed in the piglets. All pigs here had similar husbandry practices. Each treatment had 4 replicated pens with 15 pigs per pen. All diets were formulated to provide all of the nutrients to meet NRC requirements in 2012 (Table 5-1). The relative humidity and temperature of the piglet house were monitored at 60-65% and 25-28 °C, respectively. Piglets were allowed ad libitum access to feed and water throughout the experiment for 28 days.

2.2 Sample Collection

At 28d, 6 piglets from each group were chosen randomly and euthanized aseptically. Afterwards, the entire intestine was removed from each pig. Segments of the ileum and cecum flushed with saline were collected. These intestinal segments were immediately fixed in 4% paraformaldehyde solution and then embedded in paraffin for morphological examination. The luminal digesta of the ileum and cecum was collected aseptically into sterile plastic containers and stored at -80°C until processing.

2.3. Morphological examination

PAS staining was performed according to standard protocols (Shatos et al. 2003). Paraformaldehyde-fixed ileum and cecum segments taken were then dehydrated with ethanol, embedded in paraffin, and sectioned (5 μm). After dewaxing and immediately washing with distilled water for 1 min, the specimens were immersed in 0.5% periodate solution (Sigma Co.) for 5 min at room temperature in the dark. Afterward, sections were immediately washed (30 s × 2) and soaked in Schiff's solution at 37°C. After 60 min, sections were washed twice with a sulfuric acid solution then quickly rinsed with distilled water. The subsequent steps followed the routine protocols of the laboratory. The sections were examined using light microscopy. The villus length, crypt depth and the numbers of goblet cells were measured by random measurement of 10 villi and 10 measurements of the crypt per section using DS-U3 (Nikon, Japanese).

2.4. Genomic DNA Extraction

For 16S rDNA sequencing, six individuals from eight slaughtered piglets in every group were selected randomly (n = 6). Microbial DNA of digesta samples of the caecum was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA

quality was evaluated on 1% agarose gels.

2.5 Illumina Mi-seq Sequencing

To analyze the taxonomic composition of the bacterial community, the V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were performed with the following program: an initial denaturation at 95 °C for 3 min, 27 cycles of 30 s at 95 °C, annealing 55 °C for 30 s, and 45s for elongation at 72 °C, and a final extension at 72 °C for 10 min and held at 4°C. PCR reactions were performed in triplicate with a final volume of 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The PCR products were extracted using electrophoresis on 2% agarose gels and further purified with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250 bp) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols. Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) carried out the sequencing.

2.6. Bioinformatics Analysis

Raw read quality was quality-filtered using the QIIME (ver. 1.9.0, http://qiime.org/index.html) software package according to the following criteria: (i) the reads were clipped with an average quality score < 20 over a 50-bp sliding window. (ii) sequences whose overlap being longer than 10 bp were merged according to their overlap with mismatch no more than 2 bp. (iii) reads with more than two nucleotide mismatches in the primer, any mismatch in barcode, or ambiguous nucleotides were removed. The clean reads were compared with the reference database using the UCHIME algorithm to detect chimera sequences (Edgar et al. 2011). Operational taxonomic units (OTUs) were clustered with 97% similarity level using UPARSE（version 7.1 http://drive5.com/uparse/) with a novel 'greedy' algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using a confidence threshold of 70%. α-diversity indices including Chao1 value and Shannon index were determined by the Mothur software package (Schloss et al. 2009). β-diversity was investigated with QIIME using principal coordinate analysis (PCoA) based on the Bray Curtis distance matrix.

2.7. Absolute quantification of cecal specific bacteria by qPCR

The designs of primers used for absolute quantification of cecal specific bacteria Lactobacillus, Clostridium_sensu_stricto_1 and Terrisporobacter via qPCR are shown in Table 5-2. Tenfold serial dilutions of the genomic DNA of cecal samples from 10-1 to 10-6 were subjected to qPCR to generate a standard curve. The qPCR assay of standards, samples, and no-template control was performed in triplicate on an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, USA) with a 96-well format.

The 20 μL reaction mixture contained 10 μL of SYBR Premix Ex Taq (Tli RNase H Plus; 2× concentration) from TaKaRa (Shiga, Japan); 0.5 μL of each 10μmol/L primer; 7 μL of sterile DNase-free water; and 2μL of DNA. The PCR was performed under the following conditions:

95 °C for 30 s, followed by 40 cycles of 5 s at 95°C, 40 s at 60°C, and 20 s at 72°C. The fluorescence signal was acquired following the 72 °C extension phase of each cycle. Melting curve analysis was performed to check the specificity of the products followed by a cooling step performed at 95°C for 10 s, 60°C for 60s, 95°C for 15 s (ramp rate of 0.05 °C/s). PCR products that had been resolved on a 3% agarose gel were checked after ethidium bromide staining to confirm the specificity of the amplification. Data were analyzed with ABI 7500 Real-Time PCR software version 2.0.5 using the second derivate maximum method, which calculates PCR efficiency in accordance with Pfaffl (Pfaffl and M. 2001).

2.8. Composition of short chain fatty acid in the Cecum

The concentration of short chain fatty acid (SCFA)s in the cecum was measured using the method described by Erwin et al. with modifications (Erwin et al. 1961). In brief, about one gram of cecal contents were thoroughly mixed with 10 ml distilled water, incubated at 4 °C for 24 h and centrifuged at 10,000 g for 10 min at 4 °C. 0.9 ml of supernatant was mixed with 0.1 ml 25% (v/v) metaphosphoric acid and kept in the ice bath for at least 30 min. Then, the sample was centrifuged at 10,000 g for 10 min at 4 °C and 800 microliters of the sample were injected for analysis on an Agilent 6890N GC (Palo Alto, CA).

2.9. Statistical Analysis

To compare differences among different treatments, all data were subjected to ANOVA using the MIXED procedure of SAS (SAS9.4, Cary, NC, USA). Treatment means were calculated using the LSMEANS statement and means were separated using the PDIFF option. Least square means were compared using the Tukey–Kramer adjustment. The differences were considered to be statistically significant if $P \leq 0.05$ or $0.001 < P \leq 0.01$ and were considered extremely significant if the $P < 0.001$. While $0.05 < P \leq 0.1$ was considered as having a trend of difference.

3. Results

3.1. Growth Performance

To evaluate the effect of XOS on growth performance of weaned piglets, the body weight (BW) and average daily feed intake (ADFI) were measured, and average daily gain (ADG) and feed : gain ratio (F:G) were calculated (Figure 5-1). Piglets in the CTC group and XOS500 group had higher BW and higher ADG during days 1-28 than those in the CON group ($P<0.05$). Compared with the CON group, the CTC and XOS500 groups had also significantly better F:G during days 1–28 ($P < 0.05$). However, there was no significant difference between the CTC and XOS500 group for growth performance indices of weaned piglets. No difference in ADFI was observed during different dietary groups.

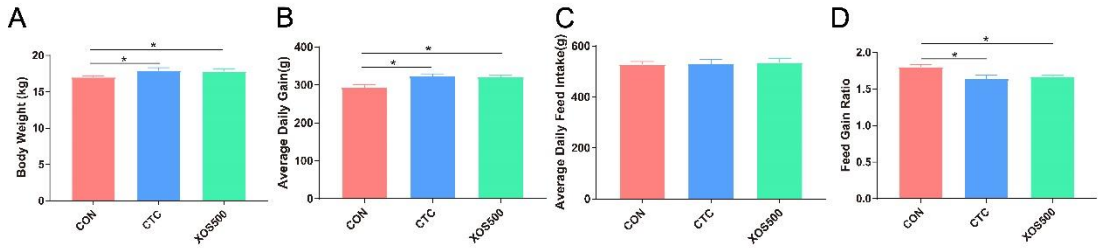


Figure 5-1. Effect of dietary treatments on growth performance in weaned piglets. (**A**) Body Weight (**B**) Average Daily Gain (**C**) Average Daily Feed Intake (**D**) Feed Gain Ratio. CON: control; CTC:

chlortetracycline; XOS500: 500mg/kg XOS. *$P \leq 0.05$.

Table 5-2. Primers used for absolute quantification of microbial populations in cecal digesta of weaned piglets.

Item	Primer (5′-3′)	Annealing temperature (°C)	Product length (bp)
Lactobacillus	Forward: GAGGCAGCAGTAGGGAATCTTC	60	118
	Reverse: CAACAGTTACTCTGACACCCGTTCTTC		
Clostridium_sensu_stricto_1	Forward: ATGCAAGTCGAGCGAKG	55	120
	Reverse: TATGCGGTATTAATCTYCCTTT		
Terrisporobacter	Forward: CGCAACCCTTGCCTTTAGT	57.5	220
	Reverse: CCCTCTGTACCACCCATTGT		

3.2. Effects of dietary treatments on ileal and cecal morphology

The effects of dietary treatments on intestinal characteristics are shown in Figure 5-2. The XOS500 supplementation increased Villus Height and Villus Height : Crypt Depth Ratio in the ileum ($P < 0.05$). Villus Height : Crypt Depth Ratio was also increased in the ileum as the CTC treatment ($P < 0.05$). Meanwhile, the XOS500 supplementation increased significantly the numbers of goblet cells in the crypt of the cecum ($P < 0.05$) compared with the CTC and CON group.

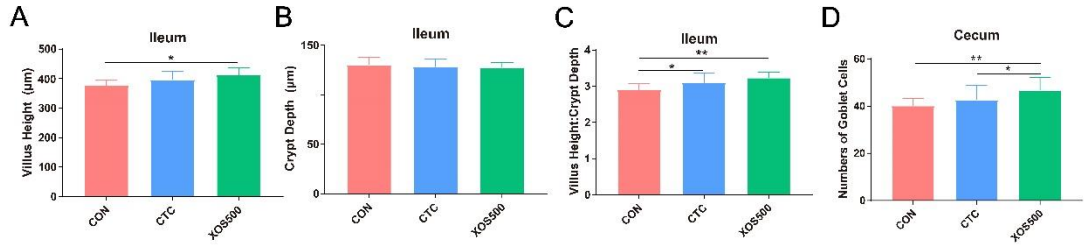


Figure 5-2. Effect of dietary treatments on histological morphology in the ileum and cecum of weaned piglets. (**A**) Villus Height (ileum) (**B**) Crypt Depth (ileum) (**C**) Villus Height : Crypt Depth (ileum) (**D**) Numbers of Goblet Cells in the crypt of Cecum. CON: control; CTC: chlortetracycline; XOS500: 500mg/kg XOS. *$P \leqslant 0.05$; **$0.001 < P \leqslant 0.01$.

3.3. DNA Sequence Analysis and Quality Filtering

A total of 1,857,463 valid sequences from 36 ileal and cecal samples remained after chimeras were filtered out and low-quality sequences were removed. Among the high-quality sequences, about 99.98% were longer than 400 bp, with an average of 433 bp. Results showed that the all Good's coverage was > 0.99, implying that most of the microbial diversity within the samples had been sufficiently captured.

3.4. Comparison between the ileal and the cecal microbiota

According to the Chao1 index and Shannon index ($P < 0.01$), there were significant differences in microbiota richness and diversity in the cecal samples compared with those in corresponding ileal samples (Figure 5-3A, B), indicating that the cecal microbiota was more diversified than the ileal microbiota. Furthermore, PCoA plots using the Bray_Curtis matrix distances, where bacterial communities clustered by the intestine, clearly showed the distinct bacterial community structure between the ileum and cecum ($R = 0.70$, $P = 0.001$, Figure 5-3C). All differential bacteria were shown in the cladogram of LEfSe in the ileum and the cecum. The circles from inner to outer represent distinct bacteria from phylum to genus levels, respectively. The yellow dots inserted in the circle suggest no significant difference in bacteria among different dietary treatments. LEfSe results showed that 38 bacterial clades at all taxonomic levels were differentially abundant (LDA > 4.0) between the ileal and cecal microbiota (Figure 5-3D).

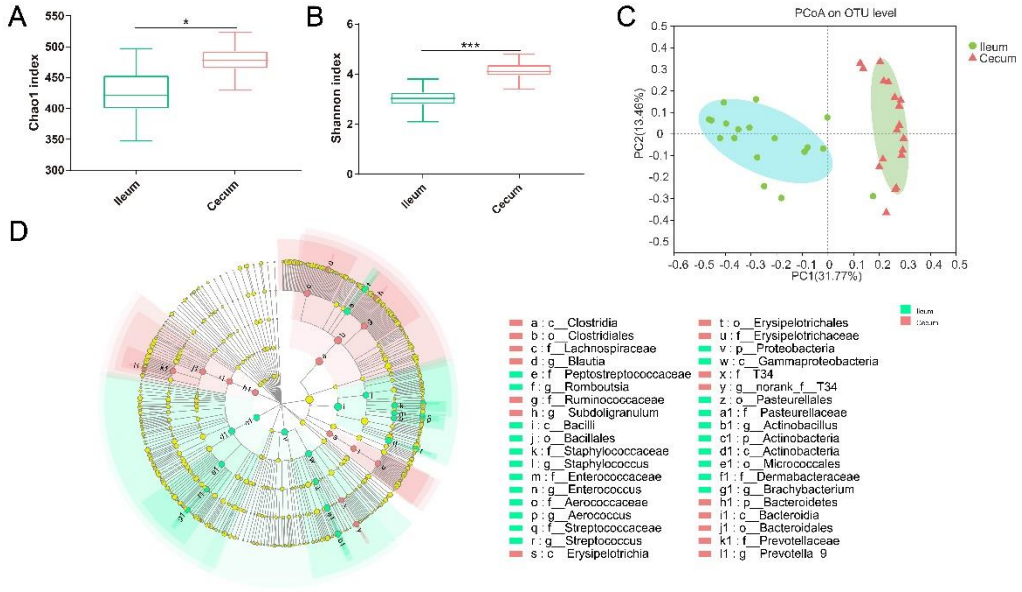


Figure 5-3. Differences between ileal and cecal microbiota of weaned piglets with supplementation XOS. Alpha diversity for ileal and cecal bacteria, for the observed **(A)** Chao1 index and **(B)** Shannon index **(C)** PCoA analysis of OTUs indicates that the bacterial profile differed strongly according to sampling site (R=0.71, *P*=0.001). **(D)** Taxa significantly associated with ileal communities (red) versus cecal communities (blue), shown in circular cladogram based on the LDA. The diameter of each circle is proportional to the abundance of the taxon. Biomarker taxa are heighted by colored circles and shaded areas. The yellow nodes indicate taxa that were not significantly differentially represented. Only differentially abundant taxa at the genus or higher taxonomic ranks were indicated.

3.5. Effects of dietary treatments on the ileal microbiota

Alpha diversity was evaluated in this study by analyzing the Chao1 index and Shannon index. Both the Chao 1 and Shannon index in the CTC group was significantly lower than the CON group. However, XOS500 supplementation significantly increased the ileal bacterial index of observed-species, the Chao1 and Shannon index (Figure 5-4A and 5-4B). Beta diversity was assessed by using the Bray_Curtis distance matrices and principal component analysis (PCoA). It was clear that the microbiota in the XOS500 group could separate from the CON group. The CTC group microbiota did not separate from the CON group community (Figure 5-4C). At the phylum level, the dominant phylum was Firmicutes in the ileum in each group (Supplementary Figure S1). At the genus level, the predominant genus was *Lactobacillus*. The relative abundance of *Lactobacillus* in the XOS500 group was significantly higher than in the CON and CTC group ($P < 0.01$), while the relative abundance of *Clostridium_sensu_stricto_1* and *Escherichia-Shigella* were remarkably reduced in the CTC and XOS500 group (Figure 5-4D).

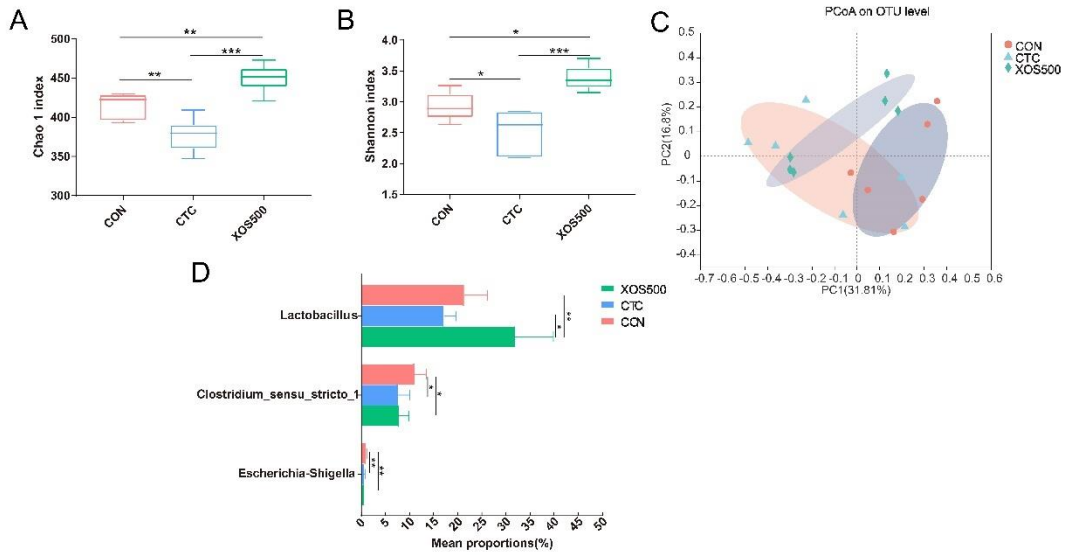


Figure 5-4. Effect of dietary treatments on ileal microbiota diversity and composition of weaned piglets. **(A)** Chao1 index **(B)** Shannon index **(C)** The beta-diversity of ileal microbiota of piglets. Principal coordinates analysis (PCoA) was used to compare the composition of ileal microbiota in different diet treatments group using Bray_Curtis. **(D)** Kruskal-Wallis H test bar plot showed the major different ileal bacterial genus during the different treatment groups. CON: control; CTC: chlortetracycline; XOS500: 500mg/kg XOS. *$P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$.

3.6. Effects of dietary treatments on the cecal microbiota

The Chao1 index and Shannon index were calculated using the OTU counts for each group and then compared among three dietary treatments (Figure 5-5A and 5-5B). The results showed that the Chao1 index and Shannon index in the XOS500 group were significantly higher than the CON and CTC group in this study. To determine similarities between microbial communities, we compared the Bray_Curtis distance of the cecum content samples from the three dietary treatments (Figure 5-5C). It was clear that the microbiota in the XOS500 group was separated from the CTC and CON group. The first axis of the PCoA explained 35.51% of the variation in bacterial diversity while the second axis explained 10.66%. No significant differences were observed with respect to the relative abundances of bacterial phyla in the cecum during these groups (Supplementary Figure S2). At the genus level, the relative abundances of *Lactobacillus* in the XOS500 group were significantly higher than the CON and CTC groups. Meanwhile, the CTC supplementation could remarkably reduce the relative abundances of *Lactobacillus* compared to the CON group. However, the piglets in the XOS500 and CTC group showed a lower relative abundance of Clostridium_sensu_stricto_1 and *Terrisporobacter* in comparison to the CON group (Figure 5-5D). The butyrate-producing genus *Blautia* and *Faecalibacterium* in the XOS500 group displayed an increasing trend compared to the CTC and CON group ($0.05 < P < 0.1$), while no significant differences were observed among the three groups (Supplementary Figure S3).

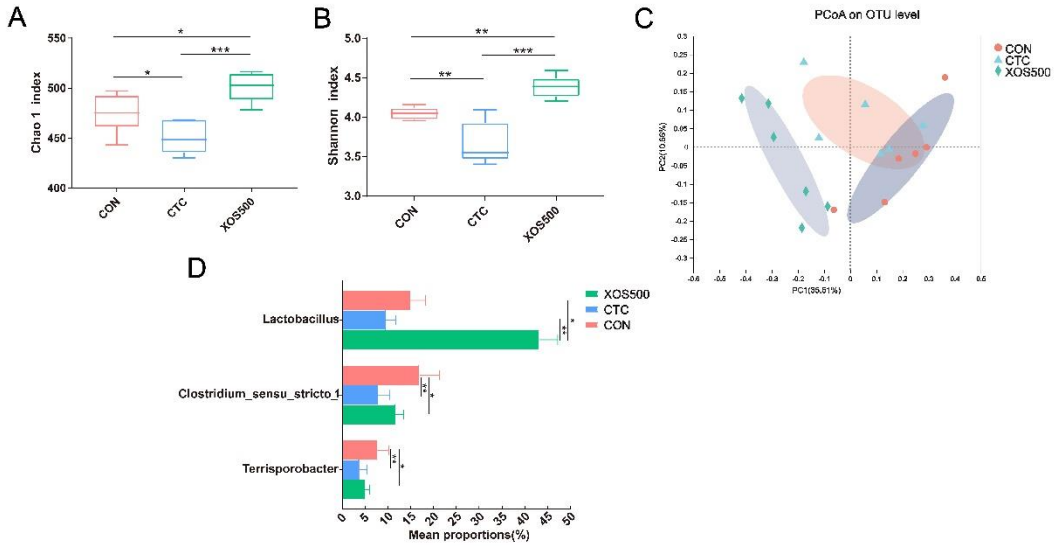


Figure 5-5. Effect of dietary treatments on cecal microbiota diversity and composition of weaned piglets. **(A)** Chao1 value **(B)** Shannon index **(C)** The beta-diversity of cecal microbiota of piglets. Principal coordinates analysis (PCoA) was used to compare the composition of cecal microbiota in different diet treatments group using Bray_Curtis. **(D)** Kruskal-Wallis H test bar plot showed the major different cecal bacterial genus during the different treatment groups. CON: control; CTC: chlortetracycline; XOS500: 500mg/kg XOS. *$P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$.

3.7. Absolute Quantification of Cecal Specific Microbiota by qPCR Assays

To determine absolute quantification of qPCR, nucleic acid standard was generated from genomic DNA of *Lactobacillus*, *Clostridium_sensu_stricto_1* and *Terrisporobacter*. The C_t-values were plotted as a function of the cell concentration and the plot showed the expected linear relationship between the copies per microliter (copies/ul) and C_t-values (**Supplementary Figure S4**). **Figure 5-6** showed that the absolute quantification of *Lactobacillus* in the XOS500 group was 7.23×10^9 copies/g cecal sample and was approximately 2.6-fold higher than the CON group (approximately 2.01×10^9 copies/g cecal sample). However, CTC supplementation remarkably reduced the absolute quantification of *Lactobacillus* (approximately 1.18×10^9 copies/g cecal sample). In addition, the XOS500 group and CTC group markedly reduced the absolute quantification of *Clostridium_sensu_stricto_1* and *Terrisporobacter* compared with the CON group ($P < 0.01$).

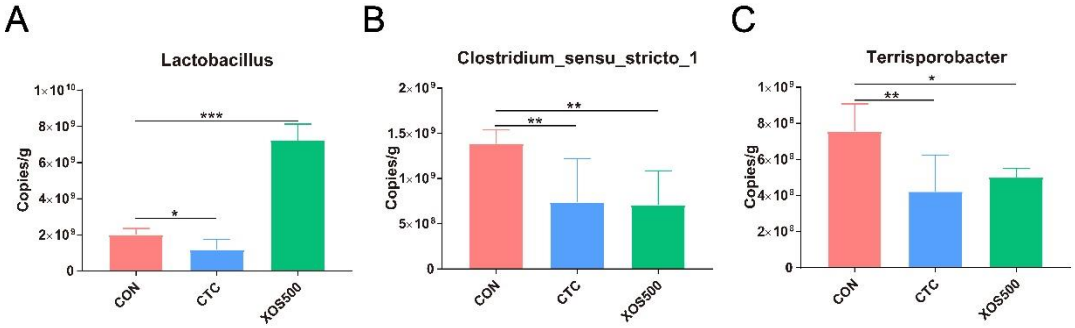


Figure 5-6. Absolute quantification for major bacteria genus on cecal samples by qPCR (copies/g sample). (**A**) Lactobacillus (**B**) Clostridium_sensus_stricto_1 (**C**) Terrisporobacter CON: control; CTC:

chlortetracycline; XOS500: 500mg/kg XOS. SEM, pooled standard error of the means; *$P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$.

3.8. SCFA in the Cecum

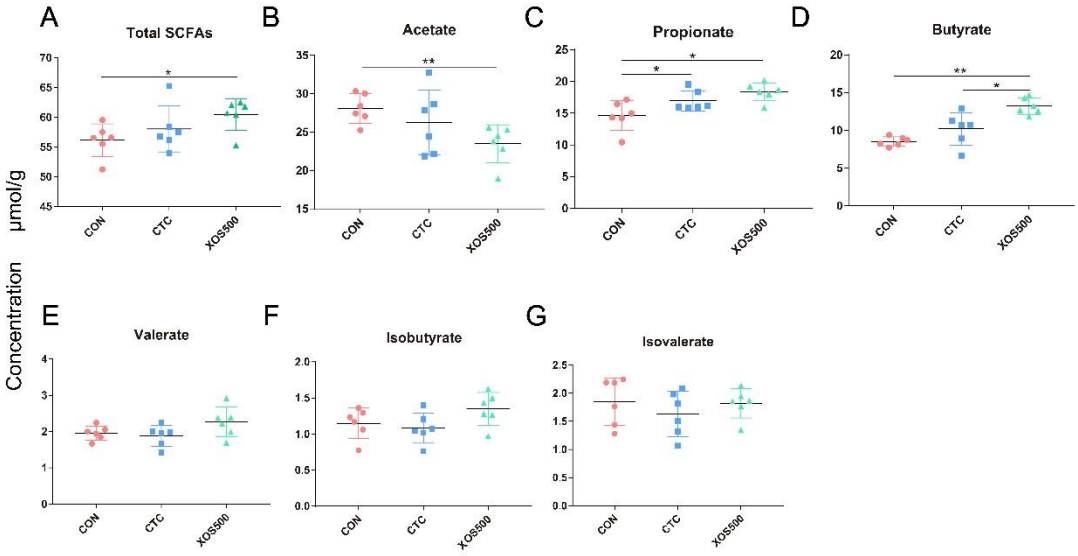


Figure 5-7. Short-chain fatty acids (SCFAs) concentration (µmol/g) in cecum of piglets during different dietary treatments. (A) Total SCFAs, (B) Acetate, (C) Propionate, (D) Butyrate, (E) Valerate, (F) Isobutyrate, (G) Isovalerate. Total SCFAs are the sum of the following SCFAs: acetate, propionate, isobutyrate, butyrate, isovalerate and valerate. Group differences were tested with a Duncan test. CON: control; CTC: chlortetracycline; XOS500: 500mg/kg XOS. *$P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$.

To further identify whether the observed microbial changes due to dietary treatment also affected the gut function, SCFA concentrations were measured. In the cecum, the most abundant SCFAs were acetate, propionate, and butyrate (Figure 5-7). Dietary supplementation with XOS500 significantly decreased the acetate concentrations and increased the total SCFAs, propionate and butyrate concentrations compared to the CON group in the cecal digesta ($P < 0.05$). In the CTC group, the concentration of propionate was remarkably higher than the CON group ($P < 0.05$). However, there is no significant difference in the SCFA concentrations between the XOS500 and CTC group.

4. Discussion

In the present study, we evaluated the effect of the administration of chlortetracycline (CTC) and xylo-oligosaccharides (XOS) on the growth performance and intestinal health of weaned piglets. We used high-throughput sequencing of the V1-V3 region of the 16S rRNA gene to monitor the ileal and cecal microbiota of piglets fed either CTC or XOS. Furthermore, we detected the absolute quantification of the specific bacterial genera and measured SCFA concentration in the cecal sample. We showed that XOS consumption altered specific bacterial genera and fermentation metabolites. We hypothesize that the improvement in growth performance of piglets is related to the characteristics of the intestinal ecosystem. Previous studies have shown that XOS are good additives for improving animal growth performance. For example, Liu et al. reported that supplemented with XOS had a greater average daily gain and feed efficiency (Liu et al. 2018). Similarly, our results showed that dietary XOS500

supplementation had a positive effect on growth performance of weaned piglets compared with the CON group. Weaned piglets fed a diet supplemented with CTC exhibited greater performance than the CON group, but no differences were observed between the CTC and XOS500 group. Some broiler studies also showed that dietary XOS significantly increased the average daily gain and reduced FCR (De Maesschalck et al. 2015; Yuan et al. 2018). In addition, Pourabedin et al. found that feed conversion ratio (FCR) in broilers fed 2 g/kg XOS diets was significantly lower than those fed CTL or 1 g/kg XOS between days 7 and 21 (Pourabedin et al. 2015). In contrast, Yin et al. reported that 0.01% XOS treatment failed to observe significant improvement in growth performance of the piglets (Yin et al. 2019). These reports indicate that different XOS doses may exert diverse effects on the growth performance of animals.

Intestinal morphology indices are often a useful criterion to estimate the nutrient digestion and absorption capacity of the intestine. Prebiotics were reported to improve growth via promoting nutrient absorption by improving intestinal structure (Pan et al. 2018). For example, Liu et al. demonstrated that XOS supplementation could improve the intestinal morphology and the apparent total tract digestibility of piglets (Liu et al. 2018). In this study, we found that villus height and villus height : crypt depth ratio of the ileum in the XOS500 treatment group was significantly increased compared to the CON group. Similarly, Maesschalck et al. found that supplementation of 0.5% XOS to the broiler feed significantly improved the villus height of the ileum (De Maesschalck et al. 2015). Villi are important structures in the small intestine, mainly involved in nutrient absorption. Therefore, an increased villus height would increase the surface area for nutrient absorption (Choe et al. 2012). These results demonstrated that XOS may improve the gut absorptive function. The possible explanation for the improvement of intestinal morphology is that XOS500 supplementation stimulated the increase of Lactobacillus. Some studies found that Lactobacillus could improve the villus height and the villus height to crypt depth ratio of the ileum in the weaned piglets (Suo et al. 2012; Yi et al. 2018). Moreover, the numbers of goblet cells in the crypt of the cecum in the XOS500 treatment group was remarkably higher than the CON group. Goblet cells produce mucin glycoproteins, constituents of the mucus, which is in the first line of defense and protects the epithelium lining the intestinal mucosa from damage and invading pathogens (Strobel et al. 2015; Li et al. 2019). The increase of goblet cells numbers thus indicates that XOS500 supplementation contributes to an improvement of the chemical barrier (mucus layer) in the cecum. This effect on the large intestinal morphology may at least partly be due to the butyrate production improvement. In our study, the increasing of butyrate-producing genus *Blautia* and *Faecalibacterium* abundance in the hindgut may positively bring about butyrate production improvement. Therefore, XOS supplementation can be a promising approach for improving intestinal morphology and protecting the intestinal barrier function in pigs.

Great variations in α-diversity and β-diversity of the microbiota were found between the ileum and the cecum, similar to what has been previously observed in pigs (Zhang et al. 2018). Furthermore, the relative abundance of certain bacterial families or genera were differentially abundant in the ileum and cecum of the piglets. For example, the relative abundance of the family *Ruminococcaceae*, the genus *Blautia* and *Prevotella_9* was higher in the cecum compared with the ileum. The spatial changes in bacterial composition along the intestinal tract may result from the dramatic changes in the intestinal microenvironments. On the one hand, from the ileum to cecum, oxygen availability significantly decreases (Espey and Graham 2013).

On the other hand, pH gradient along the intestine is another important factor to influence the dynamic composition of the microbiota (Duncan et al. 2009). In addition, most dietary nutrients are fully digested at the end of the ileum under normal physiological conditions, and undigested material is then fermented by the microbiota in the large intestine.

High bacterial diversity is beneficial for the general health and productivity of animals (Lukić et al. 2019). We used the Chao1 index and Shannon index to compare the microbial diversity among different treatment groups. The CTC group both in the ileum and cecum showed a lower alpha diversity, which is in line with other studies using an antibiotic treatment (Knecht et al. 2014; Looft et al. 2014). Meanwhile, the CTC treatment significantly reduced the relative abundance of certain genera, mainly including *Lactobacillus* and *Clostridum_sensu_stricto_1* and *Escherichia-Shigella*. This observation is in accordance with the study of Zhang et al. who reported that chlortetracycline addition reduced the piglets Escherichia-Shigella, Lactobacillus, and Streptococcus abundance in the intestinal tract (Zhang et al. 2016). In contrast, Kim et al. demonstrated that the relative abundance of the genus Lactobacillus increased due to the antimicrobial growth promoter tylosin exposure (Kim et al. 2012). In addition, our study showed that the CTC treatment markedly increased the propionate concentration compared with the CON group in the cecum. Previous research has also found that the concentration of propionate significantly increased as a result of the addition of antibiotic in broilers and was positively correlated with the change in the relative abundance of *Propionibacterium* (Pourabedin et al. 2015). As propionate is a well-known precursor of hepatic gluconeogenesis and is regarded as an inhibitor for lipogenesis, it thus seems that the improved animal performance of the CTC treatment is in part the result of the modulation of the microbiota.

The ileal and cecal microbiota was mainly composed of Firmicutes. Within this phylum, the majority belonged to the Lactobacillus genus which is consistent with previous 16S rRNA gene-based studies (Zhang et al. 2018). Our study found that the relative abundance of certain bacterial genera was altered with XOS500 supplementation in the ileum and cecum of the piglets. For instance, the relative abundances of *Lactobacillus* increased whereas *Clostridium_sensu_stricto_1* and *Escherichia_Shigella* decreased in the ileum. It was also noted that XOS500 supplementation significantly increased Lactobacillus level and reduced *Clostridium_sensu_stricto_1* and *Terrisporobacter* level by high-throughput sequencing of 16S rRNA gene amplicons in the cecum. Absolute quantification for these specific bacteria genera on cecal samples by qPCR further confirm the above results. Furthermore, this is in accordance with a recent study showing that XOS supplementation improved the lactobacilli abundance and reduced *Escherichia coli* abundance on d14 of weanling pigs (Liu et al. 2018). Similarly, a previous study reported that the cecal microbiota of HXOS-fed chickens contained significantly higher proportions of the *Lactobacillus* genus than the other dietary treatments (Pourabedin et al. 2015). Additionally, Christensen et al. also confirmed that XOS groups had higher relative abundance of *Lactobacillus spp.* than the CON group in rat cecum content (Christensen et al. 2014). In contrast, Yin et al. found that the administration of XOS to piglets markedly reduced the relative abundance of the *Lactobacillus* genus (Yin et al. 2019). *Lactobacillus* is a dominant genus within the Firmicutes phylum having beneficial effects for health including the exclusion of pathogens, immunomodulation and the production of beneficial molecules (Kravtsov et al. 2008). The high abundance of *Lactobacillus* in the XOS500 group suggested that XOS have a real potential of promoting the proliferation of beneficial bacteria in the ileum and cecum. We

hypothesize that the increase in *Lactobacillus* abundance may contribute to the improvement in the intestinal morphology and promote piglet growth. This observation is in accordance with the study of Yi et al. who reported that *Lactobacillus* reuteri LR1 improved the villus height to crypt depth ratio of the ileum in the weaned pigs (Yi et al. 2018). This positive relation between the increase of *Lactobacillus* population and improvement in weight gain has also been confirmed in other studies (Dumonceaux et al. 2006; Lin 2011). The Clostridium_sensu_stricto_1 genus has been shown to be correlated with epithelial inflammation in the intestinal mucosa (Wang et al. 2017). In addition, Terrisporobacter is a kind of emerging anaerobic pathogen and acetogenic bacterium, which can degrade various carbon sources, like xylose and cellobiose (Deng et al. 2015; Cheng et al. 2016; Groher and Weuster-Botz 2016). Interestingly, our study showed that XOS500 supplementation only decreased the opportunistic pathogenic strains *Clostridium_sensus_stricto_1*, *Escherichia_Shigella* and *Terrisporobacter* and increased the beneficial bacteria genus *Lactobacillus*. However, CTC treatment not only reduced the abundance of pathogenic strains but also decreased the abundance of the beneficial bacteria. Therefore, the results further support that XOS500 supplementation may contribute to the resistance of piglets to disease and exert a protective effect on intestine as an alternative to in-feed antibiotics of weaned piglets for maintaining favorable gut microflora composition.

Some studies have shown that SCFAs, mainly acetate, propionate and butyrate produced by gut microorganisms have health-promoting effects. Acetate can be oxidized in the tricarboxylic acid (TCA) cycle or is involved in de novo lipogenesis by conversion into to acetyl-CoA, while propionate is a well-known precursor for gluconeogenesis in the liver and is regarded as an inhibitor for lipogenesis (Den Besten et al. 2013). Butyrate is the favored energy source for large intestinal cells and the majority of this SCFA is absorbed and utilized within the large intestine (Wang et al. 2020), while acetate and propionate enter hepatic circulation in significant quantities (Den Besten et al. 2013). Except energy provision, butyrate probably plays beneficial effects on gut morphology, growth performance and anti-inflammatory under normal physiological conditions (Carney et al. 2015; Huang et al. 2017) through regulation of gene expression like proinflammatory cytokines nuclear factor kappa B (NF-κB) and interferon gamma (IFN-γ) inhibition and activation of SCFA-specific G protein-coupled receptors (Klampfer et al. 2003; Layden et al. 2013; Silva et al. 2018). Our results showed that XOS500 supplementation greatly decreased the concentration of acetate. However, the concentrations of propionate and butyrate in the XOS500 group clearly increased. Consistent with these findings, previous studies also showed that dietary XOS significantly increased the butyric acid content and decreased the concentrations of acetate (Lecerf et al. 2012; Ding et al. 2017). A remarkable increase in cecal butyrate concentration was observed as a result of XOS500 treatment and was positively correlated with the change in the relative abundance of *Blautia* and *Faecalibacterium* in the cecum. Thus, the altered SCFA concentrations were closely associated with the changes in the intestinal microbiota. Taken the results of the intestinal morphology, microbiota and the SCFA together, it is suggested that XOS are an interesting alternative to antibiotics to promote growth performance and modulate gut health in weaning piglets. However, further research is needed on the detailed mechanism of XOS on the host gut microbiota.

5. Conclusion

In conclusion, this study indicates that dietary XOS or CTC supplementation enhanced the

growth performance, improved intestinal morphology and modulated the relative abundance of specific bacteria by changing the overall microbial structure and metabolites. The increased population of *Lactobacillus* and decreased abundance of *Clostridium_sensu_stricto_1*, *Escherichia_Shigella* and *Terrisporobacter* piglets fed XOS500 might be a growth-promoting attribute. Thus, XOS may potentially function as an alternative to in-feed antibiotics in weaned piglets in modern husbandry.

6. References

Alizadeh, A., Akbari, P., Difilippo, E., Schols, H. A., Ulfman, L. H., Schoterman, M. H. C., et al. (2015). The piglet as a model for studying dietary components in infant diets: effects of galacto-oligosaccharides on intestinal functions. Brit J Nutr. 115(4): 605-618. doi: 10.1017/s0007114515004997.

Callaway, T. R., Edrington, T. S., Anderson, R. C., Harvey, R. B., Genovese, K. J., Kennedy, C. N., et al. (2008). Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim Health Res Rev. 9(2): 217-225. doi: 10.1017/s1466252308001540.

Carney, Ellen and F. (2015). Protective role of gut microbial SCFAs. Nat Rev Nephrol. 11(3): 127-127. doi: 10.1038/nrneph.2015.10.

Cheng, M. P., Domingo, M.-C., Lévesque, S. and Yansouni, C. P. (2016). A case report of a deep surgical site infection with Terrisporobacter glycolicus/T. Mayombei and review of the literature. BMC Infect Dis. 16(1). doi: 10.1186/s12879-016-1865-8.

Choe, D. W., Loh, T. C., Foo, H. L., Hair-Bejo, M. and Awis, Q. S. (2012). Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced byLactobacillus plantarumstrains. Brit Poultry Sci. 53(1): 106-115. doi: 10.1080/00071668.2012.659653.

Christensen, E. G., Licht, T. R., Leser, T. D. and Bahl, M. I. (2014). Dietary Xylo-oligosaccharide stimulates intestinal bifidobacteria and lactobacilli but has limited effect on intestinal integrity in rats. BMC Res Notes. 2014 Sep 19;7:660. doi: 10.1186/1756-0500-7-660.

Cromwell, G. L. (2002). Why and how antibiotics are used in swine production. Anim Biotechnol. 13(1): 7-27. doi: 10.1081/ABIO-120005767.

De Maesschalck, C., Eeckhaut, V., Maertens, L., De Lange, L., Marchal, L., Nezer, C., et al. (2015). Effects of xylo-oligosaccharides on broiler chicken performance and microbiota. Appl. Environ. Microbiol. 81(17): 5880-5888. doi: 10.1128/AEM.01616-15.

Den Besten, G., Lange, K., Havinga, R., van Dijk, T. H., Gerding, A., van Eunen, K., et al. (2013). Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. Am J Physiol-Gastr L. 305(12): G900-G910. doi: 10.1152/ajpgi.00265.2013.

Den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J. and Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 54(9): 2325-2340. doi: 10.1194/jlr.R036012.

Deng, Y., Guo, X., Wang, Y., He, M. and Ruan, Z. (2015). Terrisporobacter petrolearius sp. nov., isolated from an oilfield petroleum reservoir. Int J Syst Evol Micr. 65(10): 3522. doi: 10.1099/ijsem.0.000450.

Ding, X., Li, D., Bai, S., Wang, J., Zeng, Q., Su, Z., et al. (2017). Effect of dietary xylooligosaccharides on intestinal characteristics, gut microbiota, cecal short-chain fatty acids, and plasma immune parameters of laying hens. Poultry Sci. 97(3): 874-881. doi: 10.3382/ps/pex372.

Dumonceaux, T. J., Hill, J. E., Hemmingsen, S. M. and Van Kessel, A. G. (2006). Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. Appl. Environ. Microbiol. 72(4): 2815-2823. doi: 10.1128/AEM.72.4.2815-2823.2006.

Duncan, S. H., Louis, P., Thomson, J. M. and Flint, H. J. (2009). The role of pH in determining the species composition of the human colonic microbiota. Environ Microbiol. 11(8). doi: 10.1111/j.1462-2920.2009.01931.x.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27(16): 2194-2200. doi: 10.1093/bioinformatics/btr381.

Erwin, E., Marco, G. and Emery, E. (1961). Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J Dairy Sci. 44: 1768-1771. doi: 10.3168/jds.S0022-0302(61)89956-6.

Espey and Graham, M. (2013). Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. Free Radical Bio Med. 55: 130-140. doi: 10.1016/j.freeradbiomed.2012.10.554.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastro Hepat. 14(8): 491-502. doi: 10.1038/nrgastro.2017.75.

Gobinath, D., Madhu, A. N., Prashant, G., Srinivasan, K. and Prapulla, S. G. (2010). Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. Brit J Nutr. 104(1): 40. doi: 10.1017/S0007114510000243.

Gresse, R., Chaucheyras-Durand, F., Fleury, M. A., Van de Wiele, T., Forano, E. and Blanquet-Diot, S. (2017). Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. Trends Microbiol. 25(10): 851-873. doi: 10.1016/j.tim.2017.05.004.

Groher, A. and Weuster-Botz, D. (2016). Comparative reaction engineering analysis of different acetogenic bacteria for gas fermentation. J Biotechnol. 228: 82-94. doi: 10.1016/j.jbiotec.2016.04.032.

Herrero-Fresno, A., Zachariasen, C., Hansen, M. H., Nielsen, A., Hendriksen, R. S., Nielsen, S. S., et al. (2016). Apramycin treatment affects selection and spread of a multidrug-resistant Escherichia coli strain able to colonize the human gut in the intestinal microbiota of pigs. Vet Res. 47: 12. doi: 10.1186/s13567-015-0291-z.

Hu, Q., Liu, C., Zhang, D., Wang, R. and Gao, F. (2020). Effects of Low-Dose Antibiotics on Gut Immunity and Antibiotic Resistomes in Weaned Piglets. Front Immunol. 11: 903. doi: 10.3389/fimmu.2020.00903.

Huang, W., Zhou, L., Guo, H., Xu, Y. and Xu, Y. (2017). The role of short-chain fatty acids in kidney injury induced by gut-derived inflammatory response. Metabolism. 68: 20-30. doi: 10.1016/j.metabol.2016.11.006.

Kim, H. B., Borewicz, K., White, B. A., Singer, R. S., Sreevatsan, S., Tu, Z. J., et al. (2012). Microbial shifts in the swine distal gut in response to the treatment with antimicrobial growth promoter, tylosin. Proc Natl Acad Sci U S A. 109(38): 15485-15490. doi: 10.1073/pnas.1205147109.

Klampfer, L., Huang, J., Sasazuki, T., Shirasawa, S. and Augenlicht, L. (2003). Inhibition of interferon gamma signaling by the short chain fatty acid butyrate. Mol Cancer Res. 1(11): 855-862. doi: 10.1091/mbc.E02-12-0784.

Knecht, H., Neulinger, S. C., Heinsen, F. A., Knecht, C., Schilhabel, A., Schmitz, R. A., et al. (2014). Effects of beta-lactam antibiotics and fluoroquinolones on human gut microbiota in relation to Clostridium difficile associated diarrhea. PLoS One. 9(2): e89417. doi: 10.1371/journal.pone.0089417.

Kravtsov, E. G., Yermolayev, A. V., Anokhina, I. V., Yashina, N. V., Chesnokova, V. L. and Dalin, M. V. (2008). Adhesion characteristics of lactobacillus is a criterion of the probiotic choice. B Exp Biol Med+. 145(2): 232-234. doi: 10.1007/s10517-008-0058-x.

Layden, B. T., Angueira, A. R., Brodsky, M., Durai, V. and Lowe, W. L., Jr. (2013). Short chain fatty acids and their receptors: new metabolic targets. Transl Res. 161(3): 131-140. doi: 10.1016/j.trsl.2012.10.007.

Lecerf, J.-M., Dépeint, F., Clerc, E., Dugenet, Y., Niamba, C. N., Rhazi, L., et al. (2012). Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. Brit J Nutr. 108(10): 1847-1858. doi: 10.1017/s0007114511007252.

Li, Y., Lu, X., Wu, H., Xia, M., Hou, Q., Hu, W., et al. (2019). The effect of dietary supplementation of low crude protein on intestinal morphology in pigs. Res Vet Sci. 122: 15-21. doi: 10.1016/j.rvsc.2018.11.013.

Lin, J. (2011). Effect of antibiotic growth promoters on intestinal microbiota in food animals: a novel model for studying the relationship between gut microbiota and human obesity? Front Microbiol. 2: 53. doi: 10.3389/fmicb.2011.00053.

Liu, J., Cao, S., Liu, J., Xie, Y. and Zhang, H. (2018). Effect of probiotics and xylo-

oligosaccharide supplementation on nutrient digestibility, intestinal health and noxious gas emission in weanling pigs. Asian-Australas J Anim Sci. 31(10): 1660-1669. doi: 10.5713/ajas.17.0908.

Looft, T., Allen, H. K., Cantarel, B. L., Levine, U. Y., Bayles, D. O., Alt, D. P., et al. (2014). Bacteria, phages and pigs: the effects of in-feed antibiotics on the microbiome at different gut locations. The ISME journal. 8(8): 1566. doi: 10.1038/ismej.2014.12.

Lukić, I., Getselter, D., Ziv, O., Oron, O., Reuveni, E., Koren, O., et al. (2019). Antidepressants affect gut microbiota and Ruminococcus flavefaciens is able to abolish their effects on depressive-like behavior. Transl Psychiat. 9(1). doi: 10.1038/s41398-019-0466-x.

Mair, C., Plitzner, C., Domig, K. J., Schedle, K., and Windisch, W., . (2010). Impact of inulin and a multispecies probiotic formulation on performance, microbial ecology and concomitant fermentation patterns in newly weaned piglets. J Anim Physiol An N. 94(5): e164-e177. doi: 10.1111/j.1439-0396.2010.01000.x.

Mikkelsen, L. L., Jakobsen, M. and Jensen, B. B. (2003). Effects of dietary oligosaccharides on microbial diversity and fructo-oligosaccharide degrading bacteria in faeces of piglets post-weaning. Anim Feed Sci Tech. 109(1): 133-150. doi: 10.1016/S0377-8401(03)00172-X.

Mikkelsen, L. L. and Jensen, B. B. (2004). Effect of fructo-oligosaccharides and transgalacto-oligosaccharides on microbial populations and microbial activity in the gastrointestinal tract of piglets post-weaning. Anim Feed Sci Tech. 117(1-2): 0-119. doi: 10.1016/j.anifeedsci.2004.07.015.

Neuman, H., Forsythe, P., Uzan, A., Avni, O. and Koren, O. (2018). Antibiotics in early life: dysbiosis and the damage done. FEMS Microbiol Rev. 42(4): 489-499. doi: 10.1093/femsre/fuy018.

Pan, L., Farouk, M., Qin, G., Zhao, Y. and Bao, N. (2018). The Influences of Soybean Agglutinin and Functional Oligosaccharides on the Intestinal Tract of Monogastric Animals. Int J Mol Sci. 19(2). doi: 10.3390/ijms19020554.

Pfaffl and M., W. (2001). A new mathematical model for relative quantification in realtime RT-PCR. Nucleic Acids Res. 29(9): 45-45. doi: 10.1093/nar/29.9.e45.

Pourabedin, M., Guan, L. and Zhao, X. (2015). Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. Microbiome. 3: 15. doi: 10.1186/s40168-015-0079-4.

Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., Senani, S., et al. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. Bioact Carbohydr Diet Fibre. 5(1): 62-71. doi: 10.1016/j.bcdf.2014.12.003.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75(23): 7537-7541. doi: 10.1128/AEM.01541-09.

Shatos, M. A., Ri´os, J. D., Horikawa, Y., Hodges, R. R., Chang, E. L., Bernardino, C. R., et al. (2003). Isolation and Characterization of Cultured Human Conjunctival Goblet Cells. Investig Ophthalmol Vis Sci. 44(6). doi: 10.1167/iovs.02-0550.

Silva, J. P. B., Navegantes-Lima, K. C., Oliveira, A. L. B., Rodrigues, D. V. S., Gaspar, S. L. F., Monteiro, V. V. S., et al. (2018). Protective Mechanisms of Butyrate on Inflammatory Bowel Disease. Curr Pharm Des. 24(35): 4154-4166. doi: 10.2174/1381612824666181001153605.

Smith, M. G., Jordan, D., Chapman, T. A., Chin, J. C., Barton, M. D., Do, T. N., et al. (2010). Antimicrobial resistance and virulence gene profiles in multi-drug resistant enterotoxigenic Escherichia coli isolated from pigs with post-weaning diarrhoea. Vet Microbiol. 145(3-4): 299-307. doi: 10.1016/j.vetmic.2010.04.004.

Suo, C., Yin, Y., Wang, X., Lou, X., Song, D., Wang, X., & Gu, Q. (2012). Effects of lactobacillus plantarum ZJ316 on pig growth and pork quality. BMC Veterinary Research, 8(1), 89. doi:10.1186/1746-6148-8-89.

Strobel, S., Encarnação, J., Becker, N. and Trenczek, T. (2015). Histological and histochemical analysis of the gastrointestinal tract of the common pipistrelle bat (Pipistrellus pipistrellus). Eur J Histochem. 59(2). doi: 10.4081/ejh.2015.2477.

Toutain, P. L., Ferran, A. A., Bousquet-Melou, A., Pelligand, L. and Lees, P. (2016). Veterinary Medicine Needs New Green Antimicrobial Drugs. Front Microbiol. 7: 1196. doi:

10.3389/fmicb.2016.01196.
Wang, R. X., Lee, J. S., Campbell, E. L. and Colgan, S. P. (2020). Microbiota-derived butyrate dynamically regulates intestinal homeostasis through regulation of actin-associated protein synaptopodin.PNAS. 117(21): 11648-11657. doi: 10.1073/pnas.1917597117.
Wang, T., Teng, K., Liu, Y., Shi, W., Zhang, J., Dong, E., et al. (2019). Lactobacillus plantarum PFM 105 Promotes Intestinal Development Through Modulation of Gut Microbiota in Weaning Piglets. Front Microbiol. 10: 90. doi: 10.3389/fmicb.2019.00090.
Wang, W., Van Noten, N., Degroote, J., Romeo, A., Vermeir, P. and Michiels, J. (2019). Effect of zinc oxide sources and dosages on gut microbiota and integrity of weaned piglets. J Anim Physiol Anim Nutr (Berl). 103(1): 231-241. doi: 10.1111/jpn.12999.
Wang, Y., Xu, C., Zhang, R., Chen, Y., Shen, Y., Hu, F., et al. (2020). Changes in colistin resistance and mcr-1 abundance in Escherichia coli of animal and human origins following the ban of colistin-positive additives in China: an epidemiological comparative study. Lancet Infect Dis. 20(10): 1161-1171. doi: 10.1016/s1473-3099(20)30149-3.
Wang, Y., Xu, L., Liu, J., Zhu, W. and Mao, S. (2017). A high grain diet dynamically shifted the composition of mucosa-associated microbiota and induced mucosal injuries in the colon of sheep. Front Microbiol. 8: 2080. doi: 10.3389/fmicb.2017.02080.
Wijtten, P. J., van der Meulen, J. and Verstegen, M. W. (2011). Intestinal barrier function and absorption in pigs after weaning: a review. Brit J Nutr. 105(7): 967-981. doi: 10.1017/S0007114510005660.
Williams, B. A., Verstegen, M. W. and Tamminga, S. (2001). Fermentation in the large intestine of single-stomached animals and its relationship to animal health. Nutr Res Rev. 14(2): 207-228. doi: 10.1079/NRR200127.
Xun, W., Shi, L., Zhou, H., Hou, G., Cao, T. and Zhao, C. (2015). Effects of curcumin on growth performance, jejunal mucosal membrane integrity, morphology and immune status in weaned piglets challenged with enterotoxigenic Escherichia coli. Int Immunopharmacol. 27(1): 46-52. doi: 10.1016/j.intimp.2015.04.038.
Yang, J., Summanen, P., Henning, S., Hsu, M., Lam, H., Huang, J., et al. (2015). Xylooligosaccharide supplementation alters gut bacteria in both healthy and prediabetic adults: a pilot study. Front. Physiol. 6: 216. doi: 10.3389/fphys. doi: 10.3389/fphys.2015.00216.
Yi, H. B., Yang, Z. D., Xiong, Y. X., Wen, X. L., Wang, Z. L., Yand, X. F., et al. (2018). Effects of Lactobacillus reuteri LR1 on the growth performance, intestinal morphology, and intestinal barrier function in weaned pigs. J Anim Sci. doi: 10.1093/jas/sky129/4969323.
Yin, J., Li, F., Kong, X., Wen, C., Guo, Q., Zhang, L., et al. (2019). Dietary xylo-oligosaccharide improves intestinal functions in weaned piglets. Food Funct. 10(5): 2701-2709. doi: 10.1039/c8fo02485e.
Yuan, L., Li, W., Huo, Q., Du, C., Wang, Z., Yi, B., et al. (2018). Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens. PeerJ. 6: e4435. doi: 10.7717/peerj.4435.
Zhang, D., Ji, H., Liu, H., Wang, S., Wang, J. and Wang, Y. (2016). Changes in the diversity and composition of gut microbiota of weaned piglets after oral administration of Lactobacillus or an antibiotic. Appl Microbiol Biot. 100(23): 10081-10093. doi: 10.1007/s00253-016-7845-5.
Zhang, L., Wu, W., Lee, Y. K., Xie, J. and Zhang, H. (2018). Spatial Heterogeneity and Co-occurrence of Mucosal and Luminal Microbiome across Swine Intestinal Tract. Front Microbiol. 9: 48. doi: 10.3389/fmicb.2018.00048.
Zhao, P. Y., Jung, J. H. and Kim, I. H. (2012). Effect of mannan oligosaccharides and fructan on growth performance, nutrient digestibility, blood profile, and diarrhea score in weanling pigs. J Anim Sci. 90(3): 833. doi: 10.2527/jas.2011-3921.

6

General discussion and perspectives

General discussion and perspectives

Overview

In the modern swine industry, weaning is generally practiced at around 3-4 weeks of age (Jensen, 1986). Weaning stress is a sudden, stressful, short, and complex event characterized by changes in gut functions, including digestion, absorption, secretion, and immunity, which profoundly impacts piglet health and leads to decreased performance and sometimes mortality (Campbell et al., 2013). There is a direct correlation between "healthy" intestines and animal performance. A healthy gut not only regulates the intestinal physiological homeostasis, but also systemically other organ systems as well as supports the host ability to withstand environmental and infectious stressors (Gresse et al., 2017). Therefore, it is important to help piglets recover from the weaning stress and promote growth performance in the pig industry. Given the rise in public health concerns about the spread of multi-resistant bacteria using therapeutic antibiotics in gut microbiota dysbiosis, there is an urgent need for finding antibiotic alternatives to restore microbial balance and control GI infections associated with weaning transition in piglets. Prebiotics seem to have the potential as they constitute the only feed additive that is efficient towards pathogenic strains in piglets.

Therefore, the overall aim of this study was to firstly investigate the prebiotic effect of XOS on the intestinal morphology, mucosal tight junction and inflammatory response, composition of the gut microbiota, metabolic short-chain fatty acids, and on growth performance for monitoring the early life of piglets' health status with different ways. The thesis was discussed from the following points.

1. Prebiotic Effects on Intestinal Histomorphology

The gut villus structure and barrier integrity play an important role in intestinal functions. The intestinal morphology indices such as VH, CD, and VH:CD ratio are often used as a criterion to estimate the nutrient digestion and absorption capacity of the small intestine. The increase in the VH:CD ratio indicates that the effective absorption area increases and the absorption capacity becomes stronger; while the decrease in the VH:CD ratio indicates that the effective absorption area decreases and the absorption capacity weakens (Caspary, 1992; Xu et al., 2003). Moreover, villi height/crypt depth ratio increase is directly correlated with increased epithelial cell turnover, and villi height are correlated with activated cell mitosis (Samanya and Yamauchi, 2002; Chichlowski et al., 2007). Several studies have shown that the intestinal morphological characteristics are changed with supplementing prebiotics including FOS, MOS and inulin in the diet. For example, Xu et al. found that the ratio of ileum villi height and jejunum and ileum villi height to crypt depth of broiler chickens is significantly increased with 4% fructo-oligosaccharide in the diet, while the crypt depth of jejunum and ileum is significantly reduced (Xu et al., 2003). A previous study showed that the inulin-containing diet resulted in longer jejunal villi and deeper crypts than in control birds (Rehman et al., 2007). And 5% inulin can significantly extend the length of mouse ileum villi (Kim, 2002). In our study, intestinal morphology changes in the piglets provided useful information concerning xylooligosaccharide as a prebiotic. Moreover, these morphological characteristics showed a quadratic dose-dependent effect of xylo-oligosaccharide in the piglets. We found that the VH and VH:CD ratio of jejunum and ileum significantly increased in the XOS500 fed pigs compared to the CON group (Chapter 4). However, XOS100 and XOS1000 supplementation group could not significantly improve the intestinal structure. These results indicate that the optimal dose

of XOS supplementation may provide extra nutrients for healthy intestinal development. Similarly, Ding et al. demonstrated that there was a linear improvement in villus height and VH: CD ratio of the jejunum as dietary XOS concentration increased in laying hens (Ding et al., 2018). In contrast, Suo et al. found that the addition of XOS with a purity of 35% in the diet decreased the crypt depth of the duodenum in broilers (Suo et al., 2015). The possible explanation for the improvement of intestinal morphology is that XOS supplementation stimulated the increase of *Lactobacillus*. Some studies found that *Lactobacillus* could improve the villus height and the villus height to crypt depth ratio of the ileum in the weaned piglets (Yi et al., 2018).

Goblet cells in the large intestine produce and secrete abundant mucus enriched glycoprotein, which provides a protective barrier against bacteria and other harmful stimuli. Defects in this barrier contribute to chronic diseases, including colitis and cancer (Asada et al., 2012; Strobel et al., 2015). In the present study, the numbers of goblet cells in the crypt of the cecum in the XOS500 treatment group was remarkably higher than the CON group (Chapter 5). This indicates that XOS500 supplementation contributes to an improvement of the chemical barrier (mucus layer) in the cecum. This effect on the large intestinal morphology may at least partly be due to increasing butyrate production.

2. Prebiotic Effects on mucosal Tight Junction and inflammatory response

The gut barrier integrity plays a crucial role in intestinal functions, including nutrient digestibility, absorption and preventing luminal harmful molecules from penetrating the mucosa (Yin et al., 2014; Palamidi and Mountzouris, 2018). Tight junctions are the crucial components of the intestinal mucosal barrier and exert a pivotal role in the maintenance of the barrier function. They are multiple protein complexes consisting of the transmembrane proteins occludin and claudin-2 and the cytosolic protein ZO-1(Chen et al., 2021). In the present study, we found that XOS500 supplementation markedly enhanced occludin and ZO-1 mRNA levels in the ileum of weaned piglets (Chapter 4). ZO-1 serves as a scaffold protein and cross-links and anchors tight junction strand proteins. Downregulation of ZO-1 has been widely reported to increase gut permeability, which further causes gut dysfunction and host inflammation (Miao et al., 2015). This is similar to the results of Yin et al. in piglets who observed downregulation of ZO-1 with XOS treatment (Yin et al., 2019). Therefore, our results indicate that dietary supplementation with XOS enhances the intestinal barrier integrity in weaned pigs.

Gut barrier function is also tightly associated with mucosal inflammation responses. It has been reported that one of the beneficial effects of prebiotics is stimulation of immune system, which can be direct or indirect through increasing population of beneficial bacteria and their products (Shokryazdan et al., 2017). The produced SCFAs are considered an indirect effect on the immune system. The SCFAs have been reported to have positive effects on the modulation of the immune system, primarily through the inhibition of histone deacetylases and the activation of G-protein coupled receptors (Bhutia and Ganapathy, 2015). SCFA affect pro-inflammatory cytokines production (e.g., IL-6, IL-8, IL-1β and TNFα) through enhancing NF-κB activation in TLR ligand-responses in epithelial cells (Lin et al., 2015). SCFA regulate the functions of DCs, which regulate immune response depending not only on secretion of cytokines, but also on their ability to interact with T cells (Nastasi et al., 2015). The anti-inflammatory cytokines are a series of immunoregulatory molecules that control the proinflammatory cytokine responses. The IL-10 can be considered the most important anti-

inflammatory cytokine with the ability of down-regulation of proinflammatory cytokines and their receptors (Shokryazdan et al., 2017). In our study, the results showed that XOS100 and XOS500 supplementation remarkably reduced the IL-1β and IFN-γ mRNA expression. Additionally, XOS500 supplementation significantly upregulated the expression of IL-10 mRNA (Chapter 4). Similar results were also found in broiler and mice and might be caused by the increasing concentrations of SCFAs (Hansen et al., 2013; Yuan et al., 2018).

The immunoglobulins, such as IgA, IgG, IgM and sIgA, produced by plasma cells, represent an important component of the humoral immune response. Kim et al. reported that SCFAs mixture fermented from dietary fiber increased IgA expression in various compartments of the intestine as well as the levels of IgA and IgG in the blood circulation (Kim et al., 2016). In our case, the results showed that XOS500 supplementation significantly increased serum IgG concentration on d 14 and 28 compared with the CON group in the weaned piglets. Similarly, Abdelmakek et al. also reported that XOS treatment significantly increased the serum immunoglobulin level compared with the control in Dicentrarchus labrax fingerlings (Abdelmalek et al., 2015). In addition, FOS and inulin supplementation in the diet showed enhanced production of blood IgG in the mice (Cani et al., 2009).

3. Prebiotic Effects on Blood Antioxidant Activity and Immunity

Serum biochemical parameters are often used to evaluate the physiological effects of nutrients in animals. Antioxidant parameters are regarded as important serum indices to assess animals' health. Changes in the antioxidant defense system, mainly including T-AOC, T-SOD, CAT, and GSH-Px, may indicate oxidative stress (Zhu et al., 2012). The generation and elimination of free radicals are in a dynamic balance and can prevent diseases by maintaining a favorable and harmless level (Lobo et al., 2010). In our study, we found that XOS500 supplementation markedly improved antioxidant activity of weaned piglets (**Chapter 4**). the results showed that XOS500 supplementation increases of T-AOC, T-SOD, and CAT activities on days 14 and 28 compared with the CON group. In addition, the MDA level in the XOS500 group was significantly lower than that of piglets in the CON group on days 14 and 28. These results indicate that XOS supplementation had a positive effect on the antioxidant status of weaned piglets. Similarly, a previous study also reported that wheat bran xylo-oligosaccharides could increase antioxidant status in rats fed a high-fat diet (Wang et al., 2011). Additionally, Valls et al. found that the acidic XOS with MeGlcA decorations produced by GH30 named UXOS, showed a strongly higher antioxidant activity than the XOS produced by GH10 (Valls et al., 2018). Bouiche et al. also reported that UXOS showed higher antioxidant activity than AXOS. UXOS comprise neutral and acidic XOS with methylglucuronic acid (MeGlcA) ramifications, while AXOS contain only neutral molecules with arabinose decorations. The MeGlcA ramifications seem to have an important role in the antioxidant capacity of oligosaccharides. Besides, the increase of UXOS size correlates with an increase in their activity (Bouiche et al., 2019). Therefore, we speculate that UXOS may show a better antioxidant activity in weaned piglets. However, the detailed mechanism of different XOS structures on biochemical parameters needs further study.

4. Factors influencing the composition of gut microbiota of weaned piglets

In **Chapter 5**, we investigated the effects of xylo-oligosaccharides on gut microbiota as

potential replacements for antibiotic in weaning piglets. The principal coordinate analysis (PCoA) revealed that gut bacterial communities of between the ileum and the large intestine formed different clusters, suggesting that the composition of adhesive microbiota are affected by the habitat sites (Figure 5-4). Some other studies have also demonstrated that the microbial composition varied along the intestinal tract (Donaldson et al., 2016; Zhang et al., 2018). The spatial dramatic changes in gut microbiota composition along the intestinal tract may be attributed to the shifts in the intestinal microenvironments. Physiological variation between the small intestine and cecum included chemical environment and diet nutrient, as well as compartmentalized host immune activity, which are known to influence bacterial community composition. Firstly, oxygen availability significantly decreased from the ileum to the cecum (Espey, 2013). Additionally, the small intestine pH is lower, and has higher levels of oxygen and antimicrobials than the cecum. Therefore, the small intestine microbial community is dominated by fast-growing facultative anaerobes (Donaldson et al., 2016). In the cecum, microorganisms are responsible for the breakdown of otherwise 'resistant' polysaccharides that are not metabolized during transit through the small intestine. Thirdly, most of dietary nutrients are fully digested at the end of ileum under normal physiological conditions, and undigested diet-derived complex polysaccharides accumulate in the large intestine and undergo bacterial fermentation and influence microbial community composition. Unsurprisingly, the influence of diet is readily apparent in studies that profile the fecal community. A study found that completely switched between plant and animal-based diets showed that the microbiome obviously shifts with diet in the human (David et al., 2014). In the current study, we found that the administration of chlortetracycline (CTC) and xylo-oligosaccharides (XOS) remarkably improved the intestinal bacteria of weaned piglets (**Chapter 5**). The CTC group both in the ileum and cecum showed a lower alpha diversity, which is in consistent with other studies using an antibiotic treatment (Knecht et al., 2014; Looft et al., 2014). Meanwhile, the CTC treatment significantly reduced the relative abundance of certain genera, mainly including *Lactobacillus* and *Clostridum_sensu_stricto_1* and *Escherichia-Shigella*. This finding is in line with the study conducted in weaning piglets by Zhang et al. who found that chlortetracycline treatment decreased *Escherichia-Shigella*, *Lactobacillus*, and *Streptococcus* abundance in the intestinal tract of the piglets (Zhang et al. 2016). Additionally, our study found that XOS500 supplementation increased the relative abundances of *Lactobacillus* whereas *Clostridium_sensu_stricto_1* and *Escherichia_Shigella* decreased in the ileum. It was also noted that XOS500 supplementation significantly increased *Lactobacillus* level and reduced *Clostridium_sensu_stricto_1* and *Terrisporobacter* level by high-throughput sequencing of 16S rRNA gene amplicons in the cecum. Absolute quantification for these specific bacteria genera on cecal samples by qPCR further confirm the above results. Furthermore, this is in accordance with a recent study showing that XOS supplementation improved the *Lactobacillus* genus level in the animal's cecum (Christensen et al., 2014; Pourabedin et al., 2015; Liu et al., 2018). Several studies demonstrated that the main site of action of prebiotics is in the large intestine. Our results also showed that XOS have big effects in cecum and less effects in ileum of piglets. This may be mainly because the microorganisms in the small intestine are fast-growing facultative anaerobes, while the microorganisms in the large intestine are anaerobic and could digest and utilize polysaccharides. In our study, XOS could be used by the microbiota in cecum and produce SCFA, then inhibit some pathogens and increase some beneficial bacteria. This is

similar with some previous studies. Some previous studies like broilers have also the same results. Pourabedin *et al*. reported that supplementation of 2 g XOS/kg diet increased the proportion of *Lactobacillus* genus in the cecum, whereas less effects were observed in the ileum of chicken (Pourabedin et al., 2015). Our study further indicated that dietary composition is also an important factor affecting the gastrointestinal microbiota.

5. Role of the short-chain fatty acids in the prebiotic's activity

SCFAs (i.e., acetate, butyrate, and propionate) are physiologically active byproducts primarily produced from the fermentation of soluble dietary fiber with significant prebiotic effects by beneficial commensal bacteria in the large intestine (den Besten et al., 2013). It is estimated that 90% of SCFAs are absorbed from the intestinal lumen, with the majority either metabolized by colonocytes or delivered to the liver via the hepatic portal vein. Acetate can be oxidized in the tricarboxylic acid (TCA) cycle or is involved in de novo lipogenesis by conversion into to acetyl-CoA as a precursor for butyrate synthesis (den Besten et al., 2013). Propionate is readily absorbed by enterocytes, passing to the portal vein circulation, and has an important impact on gluconeogenesis in the liver and is regarded as an inhibitor for lipogenesis in many species (Bergman, 1990; Wong et al., 2006), whereas butyrate plays its role in providing an energy source for large intestinal cells and the majority of this SCFA is absorbed and utilized within the large intestine (Wang et al., 2020). Except energy provision, butyrate probably plays beneficial effects on gut morphology, growth performance, and anti-inflammatory activity under normal physiological conditions (Carney, 2015) (Huang et al., 2017). Butyrate, in particular, is able to enhance intestinal barrier function by regulating the expression of tight junction proteins, and this effect might be mediated by the activation of AMP-activated protein kinase (AMPK) or the downregulation of claudin 2 expression (Daly and Shirazi-Beechey, 2006; Peng et al., 2009). Through the production pattern diagram of SCFAs (Figure 6-1), it is found that a number of factors can influence SCFA production, including the fermentation substrate available and the composition of microorganisms (Bhatia and Yang, 2017). SCFAs lower the intestinal pH, which can promote the growth of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*. These bacteria are potent SCFA producers and play a role in maintaining healthy immune responses (LeBlanc et al., 2017). In present study, we found that XOS500 supplementation greatly decreased the concentration of acetate and increased the concentrations of propionate and butyrate (**Chapter 5**). This is in agreement with the previous studies that dietary XOS significantly increased the butyric acid content and decreased the concentrations of acetate (Lecerf et al., 2012; Ding et al., 2018). Additionally, a remarkable increasement of butyrate concentration was positively correlated with the change in the relative abundance of *Blautia* and *Faecalibacterium* in the cecum. Therefore, the results further support that altered SCFA concentrations were closely associated with the changes in the intestinal microbiota in weaned piglets.

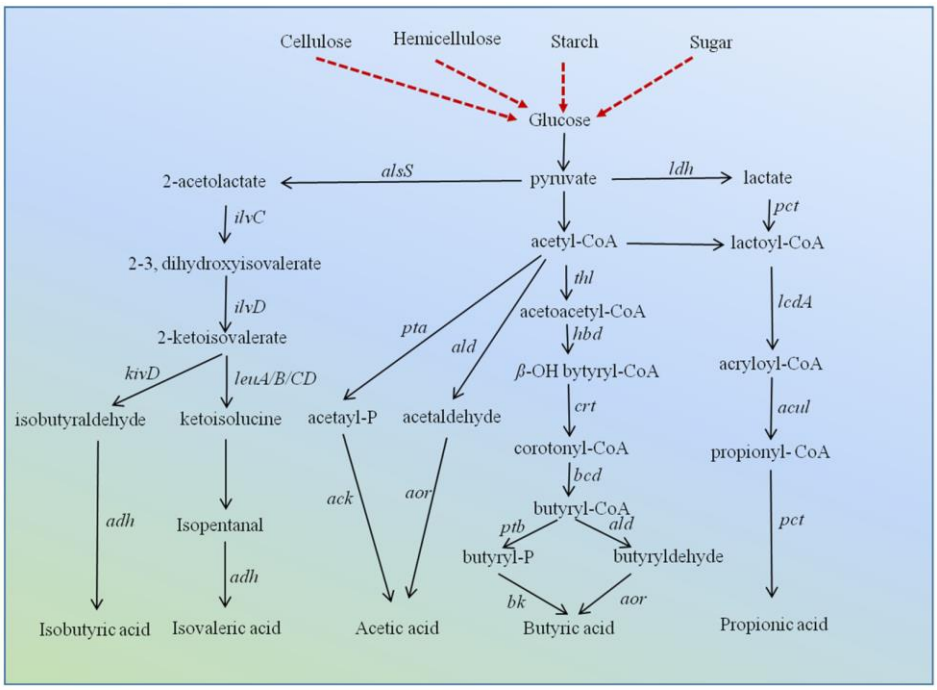


Figure 6-1. Schematic of metabolic pathways for SCFAs production (Bhatia et al., 2017)

6. The beneficial prebiotic effects on growth performance

It is known that being the potential substitute of antibiotic, prebiotics has been commonly studied in animals. It is important to stress that certain positive effects of prebiotics have frequently been observed at the weaning stage, that is the critical period for growing pigs, when animals are affected by environmental, nutritional and immunological stresses which often have a negative impact on various metabolic processes, leading to digestive disorders, diarrhoea, reduced growth performance and increased mortality. For instance, Liu et al. reported that dietary supplementation with 0.01% or 0.02% of chito-oligosaccharide (COS) positively affected FI, BWG and FCR, increased the digestibility of nutrients of weaned piglets (Liu et al., 2008). Additionally, Wan et al. showed that inclusion of alginate oligosaccharide (AOS) in the diet for 2 wk increased the average daily body weight gain in weaned pigs and have beneficial effects on the growth performance of weaned pigs (Wan et al., 2018). However, some studies reported the effect of prebiotics on host growth performance were inconsistent, which might be due to the different animal models or doses. One study found that inulin treatment reduced body weight and no significant differences in feed conversion ratio of broiler chickens as compared with control group (Sarangi et al., 2016). In our study, we observed that XOS500 significantly increased BW and ADG and decreased G:F of piglets. Even though there were no significant differences in ADFI during the different treatments, there was a numerical increase compared to the control group. A possible explanation for this result is that the intake of xylo-oligosaccharides improves the intestinal health of weaned piglets, promotes the G:F, and thus increases ADG. Our results were in line with previous studies. For example, Liu et al. reported that 200 mg/kg XOS with a purity of 50% remarkably increased ADG and feed efficiency in weaned piglets (Liu et al., 2018). Moreover, related studies showed positive dose effects of XOS on growth performance in broilers. One study demonstrated that feed conversion ratio (FCR) in broilers fed 2 g/kg XOS diets was significantly lower than those fed a control diet or 1 g/kg XOS between days 7 and 21 (Pourabedin et al., 2015). However, the different XOS dose groups

showed a quadratic effect on BW on day 28, ADG, and G:F day 1 to -28 of piglets. The XOS1000 in our study significantly failed to improve the growth performance in weaned piglets, which may be due to an excessive dose of XOS. These results on the growth performance indicate that there will be an optimal XOS dose for weaned piglets. Other oligosaccharides, such as COS have also been demonstrated to have a dose-effect affecting the growth performance of pigs (Liu et al., 2010).

7. The beneficial effects prebiotic on human

XOS were demonstrated to have various beneficial effects on human health such as inducing immune modulation, anti-tumor, antioxidant and anti-microbial activity. XOS are in great demand and have a high market value The similarity between humans and pigs in terms of intestinal microbial ecosystem and fiber fermentation capacity places the pig in a superior position over other animal models, especially for dietary fiber intervention research (Heinritz et al., 2013). The human studies revealed significant decrease of fecal pH with concomitant increase of fecal moisture and population of bifidobacterial (Samanta et al., 2015). In addition, consumption of XOS by pregnant woman is recorded to be highly effective against constipation without any other adverse effects (Tateyama et al., 2005). Moure et al. reported that XOS are used in pharmaceutical preparations due to various activities, for example, antiallergic activity, antimicrobial activity, anti-inflammatory activity, selective cytotoxicity, immunomodulatory activity (Moure et al. 2006). XOS also showed the effect of prevention of cancer and inhibition toward carcinogenesis. (Howe et al. 1992) XOS may have a significant effect on reducing blood sugar lipids eventually affecting in type 2 diabetes mellitus (Sheu et al. 2008).

8. Practical considerations of XOS

XOS are the degraded products prepared by chemical, physical or enzymatic degradation of xylan derived from biomass materials such as sugarcane residues, corn cobs, rice straw, etc (Jain et al., 2015). Generally, XOS are mixtures of oligosaccharides formed by xylose residues linked through β-(1→4)-linkages (Aachary and Prapulla 2008). In addition to xylose residues, xylan is usually found in combination with other side groups such as α-D-glucopyranosyl uronic acid or its 4-O-methyl derivative, acetyl groups, or arabinofuranosyl residues. For instance, AXOS refers to the main chain of 2 to 7 xylose residues linked by β-1,4-glycosidic bonds oligosaccharides, and the side chain α-L-Arabinofuranose is substituted at the C-2 and/or C-3 positions. AXOS are arabinose substituted XOS. XOS released by xylanases of family GH30 are all MeGlcA substituted xylooligomers UXOS. The presence of these side groups results in branched XOS with diverse biological properties. In our study, we used common linear chain XOS.

XOS are usually white or light yellow powder and no odor. In our study, we use white powder sourced from corncob by chemical degradation method and the purify is 95%. We uniformly mix XOS into the feed according to different concentrations. In the meanwhile, when we design our experiments, we chose the concentration are 100, 500 and 1000 mg/kg, respectively. On the one hand, we referred to other studies like Yin et al. used the purity of XOS is 40% and 200mg/kg and showed that produced beneficial effects (Yin et al., 2019). However, the purity of XOS in our study is 95%, and has a higher purity than other researches. We chose the first dose is 100 mg/kg, the second and third doses are 5 times and 10 times as first group in order to study the dose effects. According to our present results, we could consider to use other

concentrations like 300, 600 and 900 mg/kg XOS in the diet and will be beneficial to test a linear effect in the future researches. Supplementing 500 mg XOS per kg diet showed the best effect according to our study. The current market price of per kg a purity of 95% XOS is about 200 RMB, around 30 Euro. That is to say, per ton of feed in our study need to spend 100 RMB or 15 Euro.

In our study, we used chlortetracycline (CTC) as positive control group. The results showed that CTC treatment significantly reduced the relative bacterial abundance of certain genera in the ileum and cecum mainly including Lactobacillus and Clostridum_sensu_stricto_1 and Escherichia-Shigella. This observation is in accordance with the study of Zhang et al. who reported that chlortetracycline addition reduced the piglets Escherichia-Shigella, Lactobacillus, and Streptococcus abundance in the intestinal tract (Zhang et al. 2016). In addition, our study showed that the CTC treatment markedly increased the propionate concentration compared with the CON group in the cecum. This observation is consistent with the study of Zhang et al. who reported that chlortetracycline addition reduced the Escherichia-Shigella, Lactobacillus, and Streptococcus abundance in the piglets' intestinal tract (Zhang et al. 2016). XOS500 supplementation increased the relative abundances of Lactobacillus in the ileum and cecum of the piglets, whereas Clostridium_sensu_stricto_1 and Escherichia_Shigella decreased in the ileum. Therefore, both CTC and XOS500 treatment reduced the abundance of pathogenic strains. In the meanwhile, CTC treatment also decreased the abundance of the beneficial bacteria. Thus, XOS may potentially function as an alternative to in-feed antibiotics in weaned piglets in modern husbandry.

In addition to nutritional strategies to alleviate piglets' weaning stress and reduce the serious economic losses, we could also improve the management. Firstly, we should do a good job in the transition of feed and feeding methods. Piglets are still fed lactation feed within 2 weeks of weaning, and gradually transition to weaned piglet feed after 2 weeks. Pigs were fed in limited quantities within 5 days after weaning, and ad libitum after 5 days. Secondly, we can transition to environmental conditions by removing the sows at weaning and leaving the piglets in the pen, allowing them to live in a familiar environment. After the spirit, appetite and feces of the weaned piglets are normal, gradually change the feed, feeding system and carry out work such as stowage. When the weaned piglets are transferred to the group, the original litter piglets are generally transferred to the piglet breeding house, and they are kept in the same pen. Thirdly, adjusting the temperature and humidity of the pig house. In winter, special attention should be paid to heat preservation. When weaning, the ambient temperature can be appropriately increased to 25 to 30 °C, and at the same time, the intrusion of evil wind must be prevented. In summer, when the humidity is high, pay attention to ventilation and keep the room dry. When the weather changes suddenly, piglets are more stressed and must be cared for more carefully. Fourthly, we can train piglets to defecate at a fixed point. It will avoid humidity and pollution in the pig house environment, reduce the spread of diseases, and benefit the growth and development of piglets.

9. Conclusion and perspectives

To understand the effectiveness of prebiotic-supplemented diets of growth performance, intestinal morphology, mucosal immune response and the role of cecal microbiome with different concentration of XOS treatments induced in weaned piglets were observed. Furthermore, the intestinal parameters and SCFA concentration were altered in an XOS dose-

dependent way. The XOS500 supplementation could effectively increase the growth performance through enhancing the serum antioxidant defense system, elevating the serum IgG, improving the small intestinal structure, and maintaining intestinal barrier function in weaned piglets. In addition, to further evaluate the substitution effect of prebiotics on antibiotics, we compared the effects of XOS500 to CTC supplementation in weaned piglets. We found that XOS500 not only improved the growth performance and intestinal morphology but also modulated the relative abundance of specific bacteria by changing the overall microbial structure and metabolites. The increased population of *Lactobacillus* and decreased abundance of *Clostridium_sensu_stricto_1*, *Escherichia_Shigella* and *Terrisporobacter* piglets fed XOS500 might be a growth-promoting attribute. Therefore, XOS may potentially function as an alternative to in-feed antibiotics in weaned piglets in modern husbandry. The role played by prebiotics in gut microbiota-driven pathways via 16S rRNA approaches further elucidated how prebiotics contribute to the gut microbiota for improving weaned piglets' health. We could combine with multi-omics technologies (Proteomics, metabolomics and transcriptomics) to explore the mechanisms XOS on gut microbiota responsible for intestinal mucus function and metabolites production of weaned piglets. Further explore the specific mechanisms of XOS activating some signaling pathways like AMPK for anti-inflammatory and protein cascades and so on. We also conducted the cell experiment in vitro and study the mechanisms of XOS. Additionally, further studies may investigate XOS combined with other additives such as probiotics, administered to weaned piglets. Alternatively, we could compare the effects of XOS to other kinds of oligosaccharides on weaned piglets. In summary, this study provides theoretical references for the improvement of prebiotics utilization in weaned piglets, and promote the development of functional feed additives for swine industry.

10. References

Aachary AA, Prapulla SG. 2008. Corncob-induced endo-1,4-β-D-xylanase of Aspergillus oryzae MTCC 5154: production and characterization of xylobiose from glucuronoxylan. J Agric Food Chem 56(11):3981–88.

Abdelmalek, B.E., D. Driss, F. Kallel, M. Guargouri, H. Missaoui, S.E. Chaabouni, M.A. Ayadi and A. Bougatef, 2015. Effect of xylan oligosaccharides generated from corncobs on food acceptability, growth performance, haematology and immunological parameters of dicentrarchus labrax fingerlings. Fish Physiol Biochem, 41(6): 1587-1596.

Asada, R., A. Saito, N. Kawasaki, S. Kanemoto, H. Iwamoto, M. Oki, H. Miyagi, S. Izumi and K. Imaizumi, 2012. The endoplasmic reticulum stress transducer oasis is involved in the terminal differentiation of goblet cells in the large intestine. Journal of Biological Chemistry, 287(11): 8144-8153.

Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev, 70(2): 567-590.

Bhatia, S.K. and Y.-H. Yang, 2017. Microbial production of volatile fatty acids: Current status and future perspectives. Reviews in Environmental Science and Bio/Technology, 16(2): 327-345.

Bhutia, Y.D. and V. Ganapathy, 2015. Short, but smart: Scfas train t cells in the gut to fight autoimmunity in the brain. Immunity, 43(4): 629-631.

Bouiche C, Boucherba N, Benallaoua S, Martinez J, Diaz P, Pastor FIJ, Valenzuela SV.

Differential antioxidant activity of glucuronoxylooligosaccharides (UXOS) and arabinoxylooligosaccharides (AXOS) produced by two novel xylanases. Int J Biol Macromol. 2020 Jul 15;155:1075-1083.

Campbell, J.M., J.D. Crenshaw and J. Polo, 2013. The biological stress of early weaned piglets. J Anim Sci Biotechnol, 4(1): 19.

Cani, P.D., S. Possemiers, T. Van de Wiele, Y. Guiot, A. Everard, O. Rottier, L. Geurts, D. Naslain, A. Neyrinck, D.M. Lambert, G.G. Muccioli and N.M. Delzenne, 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving glp-2-driven improvement of gut permeability. Gut, 58(8): 1091-1103.

Carney, E.F., 2015. Acute kidney injury. Protective role of gut microbial scfas. Nat Rev Nephrol, 11(3): 127.

Caspary, W.F., 1992. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr, 55(1 Suppl): 299s-308s.

Chen, Y., Y. Xie, R. Zhong, H. Han, L. Liu, L. Chen, H. Zhang, Y. Beckers and N. Everaert, 2021. Effects of graded levels of xylo-oligosaccharides on growth performance, serum parameters, intestinal morphology, and intestinal barrier function in weaned piglets. J Anim Sci, 99(7). Available from https://www.ncbi.nlm.nih.gov/pubmed/34097723.

Chichlowski, M., W.J. Croom, F.W. Edens, B.W. McBride, R. Qiu, C.C. Chiang, L.R. Daniel, G.B. Havenstein and M.D. Koci, 2007. Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, primalac, and salinomycin. Poult Sci, 86(6): 1121-1132.

Christensen, E.G., T.R. Licht, T.D. Leser and M.L. Bahl, 2014. Dietary xylo-oligosaccharide stimulates intestinal bifidobacteria and lactobacilli but has limited effect on intestinal in rat.Pdf>.

Daly, K. and S.P. Shirazi-Beechey, 2006. Microarray analysis of butyrate regulated genes in colonic epithelial cells. DNA Cell Biol, 25(1): 49-62.

David, L.A., C.F. Maurice, R.N. Carmody, D.B. Gootenberg, J.E. Button, B.E. Wolfe, A.V. Ling, A.S. Devlin, Y. Varma, M.A. Fischbach, S.B. Biddinger, R.J. Dutton and P.J. Turnbaugh, 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature, 505(7484): 559-563.

den Besten, G., K. van Eunen, A.K. Groen, K. Venema, D.J. Reijngoud and B.M. Bakker, 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res, 54(9): 2325-2340.

Ding, X.M., D.D. Li, S.P. Bai, J.P. Wang, Q.F. Zeng, Z.W. Su, Y. Xuan and K.Y. Zhang, 2018. Effect of dietary xylooligosaccharides on intestinal characteristics, gut microbiota, cecal short-chain fatty acids, and plasma immune parameters of laying hens. Poultry Science, 97(3): 874-881.

Donaldson, G.P., S.M. Lee and S.K. Mazmanian, 2016. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol, 14(1): 20-32.

Espey, M.G., 2013. Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. Free Radic Biol Med, 55: 130-140.

Gresse, R., F. Chaucheyras-Durand, M.A. Fleury, T. Van de Wiele, E. Forano and S. Blanquet-Diot, 2017. Gut microbiota dysbiosis in postweaning piglets: Understanding the keys to health. Trends Microbiol, 25(10): 851-873.

Hansen, C.H., H. Frøkiær, A.G. Christensen, A. Bergström, T.R. Licht, A.K. Hansen and S.B. Metzdorff, 2013. Dietary xylooligosaccharide downregulates ifn-γ and the low-grade inflammatory cytokine il-1β systemically in mice. The Journal of nutrition, 143(4): 533-540.

Heinritz, S.N., R. Mosenthin and E. Weiss, 2013. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. Nutr Res Rev, 26(2): 191-209.

Howe, G.R.; Benito, E.; Castelleto, R.; Cornee, J.; Esteve, J.; Gallagher, R.P.; Iscovich, J.M.; Deng-Ao, J.; Kaaks, R.; Kune, G.A.; et al. 1992 Dietary Intake of Fiber and Decreased Risk of Cancers of the Colon and Rectum: Evidence from the Combined Analysis of 13 Case-Control Studies. J. Natl. Cancer Inst. 84, 1887.

Huang, W., L. Zhou, H. Guo, Y. Xu and Y. Xu, 2017. The role of short-chain fatty acids in kidney injury induced by gut-derived inflammatory response. Metabolism, 68: 20-30.

Jain, I., Kumar, V., and Satyanarayana, T. (2015). Xylooligosaccharides: An economical prebiotic from agroresidues and their health benefits. Indian J. Exp. Biol. 53, 131-142. PMID: 25872243.

Jensen, P., 1986. Observations on the maternal behaviour of free-ranging domestic pigs. Applied Animal Behaviour Science, 16(2): 131-142. Available from https://www.sciencedirect.com/science/article/pii/016815918690105X.

Kim, M., 2002. The water-soluble extract of chicory affects rat intestinal morphology similarly to other non-starch polysaccharides. Nutrition Research.

Kim, M., Y. Qie, J. Park and C.H. Kim, 2016. Gut microbial metabolites fuel host antibody responses. Cell Host Microbe, 20(2): 202-214. Available from https://www.ncbi.nlm.nih.gov/pubmed/27476413.

Knecht, H., S.C. Neulinger, F.A. Heinsen, C. Knecht, A. Schilhabel, R.A. Schmitz, A. Zimmermann, V.M. dos Santos, M. Ferrer, P.C. Rosenstiel, S. Schreiber, A.K. Friedrichs and S.J. Ott, 2014. Effects of β-lactam antibiotics and fluoroquinolones on human gut microbiota in relation to clostridium difficile associated diarrhea. PloS one, 9(2): e89417.

LeBlanc, J.G., F. Chain, R. Martín, L.G. Bermúdez-Humarán, S. Courau and P. Langella, 2017. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. Microb Cell Fact, 16(1): 79.

Lecerf, J.-M., F. Dépeint, E. Clerc, Y. Dugenet, C.N. Niamba, L. Rhazi, A. Cayzeele, G. Abdelnour, A. Jaruga, H. Younes, H. Jacobs, G. Lambrey, A.M. Abdelnour and P.R. Pouillart, 2012. Xylo-oligosaccharide (xos) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while xos alone only shows prebiotic properties. British Journal of Nutrition, 108(10): 1847-1858.

Lin, M.Y., M.R. de Zoete, J.P. van Putten and K. Strijbis, 2015. Redirection of epithelial immune responses by short-chain fatty acids through inhibition of histone deacetylases. Front Immunol, 6: 554.

Liu, J., S. Cao, J. Liu, Y. Xie and H. Zhang, 2018. Effect of probiotics and xylo-oligosaccharide supplementation on nutrient digestibility, intestinal health and noxious gas emission in weanling pigs. Asian-Australas J Anim Sci, 31(10): 1660-1669. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6127592/pdf/ajas-31-10-1660.pdf

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6127592/.

Liu, P., X.S. Piao, S.W. Kim, L. Wang, Y.B. Shen, H.S. Lee and S.Y. Li, 2008. Effects of chito-

oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of escherichia coli and lactobacillus in weaning pigs. J Anim Sci, 86(10): 2609-2618.

Lobo, V., A. Patil, A. Phatak, and N. Chandra. 2010. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn. Rev. 4:118–126.

Looft, T., H.K. Allen, B.L. Cantarel, U.Y. Levine, D.O. Bayles, D.P. Alt, B. Henrissat and T.B. Stanton, 2014. Bacteria, phages and pigs: The effects of in-feed antibiotics on the microbiome at different gut locations. Isme j, 8(8): 1566-1576.

Miao, Y.S., Y.Y. Zhao, L.N. Zhao, P. Wang, Y.H. Liu, J. Ma and Y.X. Xue, 2015. Mir-18a increased the permeability of btb via runx1 mediated down-regulation of zo-1, occludin and claudin-5. Cell Signal, 27(1): 156-167.

Moure, A.; Gullon, P.; Domınguez, H.; Parajo, J.C. 2006. Advances in the Manufacture, Purification and Applications of XyloOligosaccharides as Food Additives and Nutraceuticals. Proc. Biochem. 41, 1913–1923.

Nastasi, C., M. Candela, C.M. Bonefeld, C. Geisler, M. Hansen, T. Krejsgaard, E. Biagi, M.H. Andersen, P. Brigidi, N. Ødum, T. Litman and A. Woetmann, 2015. The effect of short-chain fatty acids on human monocyte-derived dendritic cells. Sci Rep, 5: 16148.

Palamidi, I. and K.C. Mountzouris, 2018. Diet supplementation with an organic acids-based formulation affects gut microbiota and expression of gut barrier genes in broilers. Anim Nutr, 4(4): 367-377.

Peng, L., Z.R. Li, R.S. Green, I.R. Holzman and J. Lin, 2009. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of amp-activated protein kinase in caco-2 cell monolayers. J Nutr, 139(9): 1619-1625.

Pourabedin, M., L. Guan and X. Zhao, 2015. Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. Microbiome, 3: 15. Available from https://www.ncbi.nlm.nih.gov/pubmed/25874109.

Rehman, H., C. Rosenkranz, J. Böhm and J. Zentek, 2007. Dietary inulin affects the morphology but not the sodium-dependent glucose and glutamine transport in the jejunum of broilers. Poult Sci, 86(1): 118-122.

Samanya, M. and K.E. Yamauchi, 2002. Histological alterations of intestinal villi in chickens fed dried bacillus subtilis var. Natto. Comp Biochem Physiol A Mol Integr Physiol, 133(1): 95-104.

Samanta, A.K., N. Jayapal, C. Jayaram, S. Roy, A.P. Kolte, S. Senani and M. Sridhar, 2015. Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. Bioactive Carbohydrates and Dietary Fibre, 5(1): 62-71.

Sarangi, N.R., L.K. Babu, A. Kumar, C.R. Pradhan, P.K. Pati and J.P. Mishra, 2016. Effect of dietary supplementation of prebiotic, probiotic, and synbiotic on growth performance and carcass characteristics of broiler chickens. Vet World, 9(3): 313-319.

Sheu, W.H.; Lee, I.T.; Chen, W.; Chan, Y.C. 2008. Effects of Xylooligosaccharides in Type 2 Diabetes Mellitus. J. Nutr. Sci. Vitaminol. 54, 396.

Shokryazdan, P., M. Faseleh Jahromi, B. Navidshad and J.B. Liang, 2017. Effects of prebiotics on immune system and cytokine expression. Med Microbiol Immunol, 206(1): 1-9. Available from https://www.ncbi.nlm.nih.gov/pubmed/27704207.

Strobel, S., J. Encarnação, N. Becker and T. Trenczek, 2015. Histological and histochemical

analysis of the gastrointestinal tract of the common pipistrelle bat (pipistrellus pipistrellus). European journal of histochemistry: EJH, 59(2).

Suo, H.Q., l. Lu and G.H. Xu, 2015. Effectiveness of dietary xylo-oligosaccharides for broilers fed a conventional corn-soybean meal diet.

Tateyama, I., Hashii, K., Johno, I., Iino, T., Hirai, K., Suwa, Y., et al. (2005). Effect of xylooligosaccharide intake on severe constipation in pregnant women. Journal of Nutrition Science and Vitaminology, 51, 445–448.

Valls, C., F.I.J. Pastor, T. Vidal, M.B. Roncero, P. Diaz, J. Martinez and S.V. Valenzuela, 2018. Antioxidant activity of xylooligosaccharides produced from glucuronoxylan by xyn10a and xyn30d xylanases and eucalyptus autohydrolysates. Carbohydr Polym, 194: 43-50.

Wan, J., J. Zhang, D. Chen, B. Yu, X. Mao, P. Zheng, J. Yu, J. Luo and J. He, 2018. Alginate oligosaccharide-induced intestinal morphology, barrier function and epithelium apoptosis modifications have beneficial effects on the growth performance of weaned pigs. J Anim Sci Biotechnol, 9: 58.

Wang, J., Y. Cao, C. Wang, and B. Sun. 2011. Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet. Carbohyd. Polym. 86(3):1192–1197.

Wang, R.X., J.S. Lee, E.L. Campbell and S.P. Colgan, 2020. Microbiota-derived butyrate dynamically regulates intestinal homeostasis through regulation of actin-associated protein synaptopodin. Proc Natl Acad Sci U S A, 117(21): 11648-11657.

Wong, J.M., R. de Souza, C.W. Kendall, A. Emam and D.J. Jenkins, 2006. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol, 40(3): 235-243.

Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang, 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci, 82(6): 1030-1036.

Yi, H., L. Wang, Y. Xiong, X. Wen, Z. Wang, X. Yang, K. Gao and Z. Jiang, 2018. Effects of lactobacillus reuteri lr1 on the growth performance, intestinal morphology, and intestinal barrier function in weaned pigs. J Anim Sci, 96(6): 2342-2351.

Yin, J., F. Li, X. Kong, C. Wen, Q. Guo, L. Zhang, W. Wang, Y. Duan, T. Li, Z. Tan and Y. Yin, 2019. Dietary xylo-oligosaccharide improves intestinal functions in weaned piglets. Food Funct, 10(5): 2701-2709.

Yin, J., W. Ren, J. Duan, L. Wu, S. Chen, T. Li, Y. Yin and G. Wu, 2014. Dietary arginine supplementation enhances intestinal expression of slc7a7 and slc7a1 and ameliorates growth depression in mycotoxin-challenged pigs. Amino Acids, 46(4): 883-892.

Yuan, L., W. Li, Q. Huo, C. Du, Z. Wang, B. Yi and M. Wang, 2018. Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens. PeerJ, 6: e4435.

Zhang, D., Ji, H., Liu, H., Wang, S., Wang, J. and Wang, Y. (2016). Changes in the diversity and composition of gut microbiota of weaned piglets after oral administration of Lactobacillus or an antibiotic. Appl Microbiol Biot. 100(23): 10081-10093.

Zhang, L., W. Wu, Y.K. Lee, J. Xie and H. Zhang, 2018. Spatial heterogeneity and co-occurrence of mucosal and luminal microbiome across swine intestinal tract. Front Microbiol, 9: 48. Available from https://www.ncbi.nlm.nih.gov/pubmed/29472900.

Zhu, L. H., K. L. Zhao, X. L. Chen, and J. X. Xu. 2012. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. J. Anim. Sci. 90: 2581–

2589.

Appendix-Publications

1. **Chen, Y.,** Y. Xie, K.M. Ajuwon, R. Zhong, T. Li, L. Chen, H. Zhang, Y. Beckers and N. Everaert, 2021. Xylo-oligosaccharides, preparation and application to human and animal health: A review. Front Nutr, 8: 731930. **(Published)**

2. **Chen, Y.,** Y. Xie, R. Zhong, H. Han, L. Liu, L. Chen, H. Zhang, Y. Beckers and N. Everaert, 2021. Effects of graded levels of xylo-oligosaccharides on growth performance, serum parameters, intestinal morphology, and intestinal barrier function in weaned piglets. J Anim Sci, 99(7). **(Published)**

3. **Chen, Y.,** Y. Xie, R. Zhong, L. Liu, C. Lin, L. Xiao, L. Chen, H. Zhang, Y. Beckers and N. Everaert, 2021. Effects of xylo-oligosaccharides on growth and gut microbiota as potential replacements for antibiotic in weaning piglets. Front Microbiol, 12: 641172. **(Published)**