UNIVERSITI PUTRA MALAYSIA

EFFECT OF INULIN AND FERMENTED FEED ADDITIVE ON GROWTH AND NITROGEN BALANCE IN PIGS

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FP 2005 32

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By

WANG WEISHAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

February 2005

Dedicated to

My beloved Alice

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

EFFECT OF INULIN AND FERMENTED FEED ADDITIVE ON GROWTH AND NITROGEN BALANCE IN PIGS

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February 2005

Chairman: Associate Professor Loh Teck Chwen, Ph D

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Three experiments were conducted to determine the dietary crude protein (CP) level, inulin, and local available fermented feed additive (FFA) on growth, nitrogen (N) balance and fecal characteristics in growing pigs. Experiment 1 studied the fecal microflora fermentation using inulin compared with CMC *in vitro*. Gas volume, total volatile fatty acids (VFA) and purine bases (PB) concentrations of inulin fermentation were higher ($P<0.05$) than the CMC.

Experiment 2 investigated the dietary CP level and addition of inulin on growth, nitrogen balance and fecal characteristics in growing pigs. Twenty-four crossbred barrows (Duroc x Large white x Landrace) of an average body weight of 40 kg were used to conduct a 28-day experiment. The diet treatments were two levels of CP, 18% and 14% with or without 0.3% inulin addition. Daily live weight gain (DLWG) and feed conversion ratio (FCR) were not affected ($P>0.05$) by the dietary treatments. However, N intake and N excretion were decreased ($P<0.05$) with reduced CP level. Addition of inulin without further effect on the total amount of N excretion, but tended to shift N excretion from urine to feces. Higher ($P<0.05$) lactic acid bacteria

(LAB) and lower ($P<0.05$) *Enterobacteriaceae* counts in feces for pigs fed with 14% CP and 14% CP + 0.3% inulin were observed.

The hypothesis that addition of inulin and FFA would affect gastrointestinal (GIT) microorganisms, hence to improve N utilization was validated in Experiment 3. Twenty-four crossbred barrows (Duroc x Large White x Landrace) of an average body weight 65 kg were used in the 28-day experiment. Dietary treatments were addition of 0.3% inulin (IN), 4% fermented feed additive (FFA), or their combination (IN + FFA). Pigs fed with IN + FFA had a higher ($P<0.05$) DLWG and a lower ($P<0.05$) FCR compared with other treatment groups. The quantity of total N excretion was not significantly ($P>0.05$) different among treatment groups, however, N excretion pattern tended to shift from urinary N excretion to fecal N excretion ($P>0.05$). Higher ($P<0.05$) LAB and lower ($P<0.05$) *Enterobacteriaceae* counts in feces for pigs fed with IN, FFA and IN + FFA compared to the pigs fed control diet were observed.

It is concluded that inulin is readily fermented by GIT microbes. The inclusion of inulin or fermented feed additive, or both in the diets cannot reduce total N excretion but they can shift N excretion from urine to feces in growing pigs. N excretion can be decreased by reducing dietary protein from 18% to 14% without affecting the DLWG and FCR of growing pigs. The addition of inulin with fermented feed additive in the diet can improve DLWG and FCR. Reducing dietary CP level, inclusion of inulin or fermented feed additive, or both in the diets can modify GIT microorganism toward to a beneficially balance. In summary, inulin and fermented feed additive inclusion, along with the manipulation of dietary protein levels in pig diet, is a viable avenue to reduce nitrogen excretion in growing pigs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN INULIN DAN MAKANAN ADITIF FERMENTASI YANG TELAH DI FERMENTASI KE ATAS PERTUMBUHAN DAN IMBANGAN NITROGEN PADA BABI PEMBESAR

Oleh

WANG WEISHAN

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Tiga eksperimen telah dijalankan untuk mengkaji tahap protein kasar (CP) dalam diet, inulin dan penambah makanan fermentasi tempatan (FFA) pada tumbesaran, keseimbangan nitrogen (N) dan ciri-ciri najis pada khinzir yang sedang membesar. Eksperimen 1 mengkaji fermentasi mikroflora najis dengan menggunakan inulin berbanding dengan CMC secara *in vitro*. Isipadu gas, jumlah asid lemak meruap (VFA) dan kepekatan purine bases fermentasi inulin adalah lebih tinggi ($P<0.05$) daripada CMC.

Eksperimen 2 mengkaji tahap CP diet dan penambahan inulin pada tumbesaran, keseimbangan nitrogen dan ciri-ciri najis pada khinzir yang sedang membesar. Dua puluh empat ekor khinzir jantan kacukan (Duroc x Large White x Landrace) yang telah dikembiri dengan berat purata 40 kg telah digunakan untuk eksperimen selama 28 hari. Rawatan diet adalah dua tahap CP, 18% dan 14% dengan atau tiada 0.3% penambahan inulin. Penambahan berat hidup harian dan nisbah pertukaran makanan tidak dipengaruhi ($P>0.05$) Penambahan inulin tiada pengaruh lanjut pada jumlah pengumuhan N, tetapi bercenderung memindah pengumuhan dari urin ke najis.

Bilangan bakteria asid laktik (LAB) yang lebih tinggi (*P>0.05*) dan bilangan *Enterobacteriaceae* yang lebih rendah (*P<0.05*) dalam najis khinzir yang diberi makanan 14% CP dan 14% CP + 0.3% inulin diperhatikan.

Hipotesis bahawa penambahan inulin dan FP dapat mengaruhi mikroorganisma gastrointestin, dengan itu memanfaatkan penggunaan N disahkan dalam eksperimen 3. Dua puluh empat ekor khinzir jantan kacukan (Duroc x Large White x Landrace) yang telah dikembiri dengan berat purata 65 kg telah digunakan untuk eksperimen selama 28 hari. Rawatan diet adalah penambahan 0.3% inulin, 4% FP, atau kombinasi (IN + FP). Penambahan berat hidup harian meningkat (*P<0.05*) dan nisbah pertukaran makanan yang lebih rendah (*P<0.05*) didapati untuk khinzir yang diberi makanan IN + FP berbanding dengan kumpulan rawatan lain. Kuantiti pengumuhan N tidak berbeza secara signifikan (*P>0.05*) antara diet tetapi pola pengumuhan N bercenderung berpindah dari pengumuhan urin N ke najis N (*P>0.05*). Bilangan bakteria asid laktik (LAB) yang lebih tinggi (*P<0.05*) dan bilangan *Enterobacteriaceae* yang lebih rendah (*P<0.05*) diperhatikan dalam najis khinzir yang diberi makanan inulin, FP dan IN + FP berbanding dengan khinzir diberi diet kawalan.

Ia dapat disimpulkan bahawa inulin bersedia difermentasi oleh mikrobial gastrointestin. Penambahan inulin atau produk fermentasi, atau kedua-duanya dalam diet tidak merendahkan jumlah pengumuhan N tetapi berupaya memindah pengumuhan N dari urin ke najis dalam khinzir yang sedang membesar. Pengumuhan nitrogen dapat direndahkan dengan menurunkan protein dalam diet dari 18% ke 14% dengan tidak mempengaruhi penambahan berat hidup harian dan nisbah pertukaran makanan pada khinzir yang sedang membesar. Penambahan inulin dengan penambah makanan fermentasi dalam diet dapat mamanfaatkan penambahan berat hidup harian dan nisbah pertukaran makanan. Penurunan tahap CP dalam diet, penambahan inulin atau penambah makanan fermentasi atau kedua-duanya sekali dalam diet dapat mengubah mikroorganisma dalam GIT ke arah keseimbangan yang memanfaatkan. Kesimpulannya, inulin dan penambahan makanan fermentasi berhubung dengan manipulasi tahap protein dalam diet khinzir merupakan kaedah yang sesuai untuk merendahkan pengumuhan nitrogen dalam khinzir yang sedang membesar.

ACKNOWLEGEMENTS

I would like to express my deep appreciation and sincere gratitude to my supervisor Associate Professor Dr. Loh Teck Chwen for his guidance, encouragements and advice throughout my studies. My deep appreciation is also extended to my co-supervisors Associate Professor Dr. Liang Juan Boo and Dr. Goh Yong Meng for their advice, suggestions and comments during my study. I am also indebted to Professor Dr. Liao Xin Di, Dr. Chen Xue Bin and Dr. He Dan Ling for their encouragement and suggestions. Thanks also due to Associate Professor Dr. Foo Hooi Ling for allowing me to use the laboratory facility.

I wish to acknowledge the support given by the owner of Tan Brother Pig Farm at Tanjung Sepat, Negeri Sembilan, Malaysia where I conducted the experiments. I also thank the technical staffs of Nutrition Laboratory, Department of Animal Science, Universiti Putra Malaysia.

Thanks are also due to Ms Elizabeth Law, Ms Lim Yin Sze and Ms Yap Sia Yen for their help, particularly in teaching me the necessary laboratory techniques. I would like to thank Ms Lai Pui Wah who translated the abstract into the Bahasa Malaysia. I would like to thank all my fellow students of UPM, especially Ms Wang Yan for her help and encouragement during my study.

Finally, my deepest gratitude to my parents, Wang Guo Fei and Shen Xiu Rong, sister, Wang Bao Lan, brother in law, Lin Zhao Rong for their understanding, financial and moral support during the study.

I certify that an Examination Committee met on 24th February 2005 to conduct the final examination of Wang Weishan on his Master of Science thesis entitled "Effect of Inulin and Fermented Feed Additive on Growth and Nitrogen Balance in Pigs" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

WANG WEISHAN

Date: 9 MAC 2005

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ABBREVIATIONS

AA	amino acid
CFU	colony forming units
CON	control
CP	crude protein
DE	digestible energy
DM	dry matter
FE	fecal energy
FF	fermented fruits
FFA	fermented feed additive
g	gram
GC	gas chromatography
GE	gross energy
h	hour
I	inulin
IN	inulin
kcal	kilo calorie
L	litter
ME	metabolize energy
mg	milligram
ml	milliliter
mM	millimole
N	nitrogen
NE	net energy

NEm	net energy for maintenance
NEp	net energy for production
PB	purine bases
SEM	standard error mean
TN	total Kjeldahl nitrogen
UE	urinary energy
VFA	volatile fatty acid

CHAPTER 1

INTRODUCTION

Present pig production has become highly industrialized and concentrated. The production system faces challenges of excess nutrient excretions, particularly nitrogen, which has polluted the environment. It has been noted that excessive nitrogen excretion from pig production adversely influences surrounding water quality (both surface and groundwater) and has been deemed responsible for acid rain. This has increasingly aroused public concern and expedient means for minimizing nitrogen excretion are needed urgently.

The amount of nitrogen excreted by pigs is affected by three main factors: (1) the amount of dietary nitrogen (protein) consumed, (2) the efficiency of nitrogen is utilization for growth and other functions, and (3) the amount of endogenous secretions. Generally, little can be done to influence the amount of endogenous losses (Richert and Sutton, 1997). Thus, in order to reduce the amount of nitrogen excreted by pigs, the amount consumed must be decreased, and/or the efficiency of utilization of the dietary nitrogen must be increased.

Dietary manipulation for example by reducing the crude protein content of swine diets fortify with synthetic amino acids to meet the actual needs of the pig has been reviewed (Kornegay and Verstegen, 2001). Based on a review of several papers, Kerr and Easter (1995) suggested that for each one percentage unit reduction in dietary crude protein combined with amino acid supplementation, total nitrogen losses (fecal and urinary) could be reduced by approximately 8%. However, it is presently more cost-effective to use supplemental amino acids, as most of synthetic amino acids are too expensive to use in the practical diets. Reducing dietary nitrogen input has shown a reduction in performance and an increase in backfat accretion (Kerr *et al.*, 2003; Figueroa *et al.*, 2000).

The gastrointestinal tract (GIT) of pig harbors numerically dense and metabolically active microorganisms (Gaskins 2001). The commensal microorganisms of the pig are viewed typically as a beneficial entity for the host. For example, indigenous gut bacteria provide the host with nutrients, including volatile fatty acid, vitamin K, B vitamins, and amino acids (Savage, 1986). The GIT microorganisms are affecting nitrogen digestibility in pigs (Low *et al.*, 1978; Caine *et al.*, 1999). The research interest in the nitrogen nutrition related with GIT microorganisms of the pig has been focused on their synthesis of amino acids (Fuller and Reeds, 1998; Caine *et al.*, 1999; Torrallardona *et al.*, 2003a; 2003b). However, the extent to which microbial protein contributes to the amino acid needs of the pig is unclear (Gaskins, 2001). Nevertheless, approaches to improve GIT ecosystem, by which to enhance nitrogen metabolism have aroused researchers' attention.

Inulin is fermentable carbohydrates (Flamm *et al.*, 2001), which also has been defined as prebiotic (Flickinger *et al.*, 2003). Dietary fermentable carbohydrates have shown their influences on shifting nitrogen excretion and reducing ammonia emission of pigs (Nahm, 2003). A study of Remesy and Demigne (1989) showed increased blood urea transport to the cecum and enhanced ammonia absorption in rats fed soluble fiber sources. Besides, feeding inulin to rats caused a net retention of nitrogen in cecum shifting nitrogen excretion from urine to feces. However, ileal and fecal nitrogen excretion in pigs as well as nitrogen retention were not influenced by inulin consumtion (Vanhoof and Schrijver, 1996b).

Besides, fermented feed additive consisted of locally available fruits such as lime with mixture of lactobacilli cultures as additive in the diet on growth performance, *Enterobacteriaceae* and lactic acid produce bacteria (LAB) counts in feces of the post-weaning piglets has been studied (Loh *et al.*, 2003b). In the study of Loh *et al.* (2003b), the use of fermented feed additive could significantly ($P<0.05$) reduce *Enterobacteriaceae* population in piglets' feces compared to the use of normal feed with or without antibiotic. Meanwhile, the LAB population in the feces was increased in those piglets fed with diets added with fermented feed additive (Loh *et al.*, 2003b). However, the effect of the fermented feed additive on growth, nitrogen balance and fecal characteristics in growing pigs has not been previously reported.

Therefore, base on above information, the objectives of thesis were to study dietary protein, inulin and fermented feed additive on growth, nitrogen balance and fecal characteristics in growing pigs. Three experiments were conducted to achieve the above objectives:

(i) *In vitro* study of fecal microflora fermentation using inulin.

(ii) Effects of dietary protein and inulin addition on growth, nitrogen balance and fecal characteristics in growing pigs.

(iii) Effects of feeding inulin and fermented feed additive on growth, nitrogen balance and fecal characteristics in growing pigs