ผลของขนาดอนุภาคถั่วเหลืองไขมันเต็ม ต่อคุณลักษณะการเจริญเติบโต และการย่อยได้ของสารอาหารในสุกรหลังหย่านม

นางสาวชนิกานต์ ชิ้นปิ่นเกลียว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF FULL FAT SOYBEAN PARTICLE SIZES ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN POSTWEANING PIGS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	Effects of full fat soybean particle sizes on growth performance and nutrient digestibility in weaning pigs
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ชนิกานต์ ชิ้นปิ่นเกลียว : ผลของขนาดอนุภาคถั่วเหลืองไขมันเต็ม ต่อคุณลักษณะการเจริญเติบโต และการย่อยได้ของสารอาหารในสุกรหลังหย่านม. (EFFECTS OF FULL FAT SOYBEAN PARTICLE SIZES ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN POSTWEANING PIG) อ. ที่ปรึกษา : รศ.สุวรรณา กิจภากรณ์, อ. ที่ปรึกษาร่วม : ผศ.ดร.ณัฐชนก อมรเทวภัทร, 55 หน้า.

การวิจัยครั้งนี้ประกอบด้วย 2 การทดลองเพื่อศึกษาผลของขนาดอนุภาคถั่วเหลืองไขมันเต็มต่อคุณลักษณะทางกายภาพ ต้นทุนการผลิต สารต้านโภชนะ การย่อยได้ของสารอาหาร และคุณลักษณะการเจริญเติบโตในสุกรหลังหย่านม ถั่วเหลืองไขมันเต็มขนาดอนุภาคแตกต่างกันได้จากการนำเมล็ดถั่วเหลืองผ่านเครื่องบดแบบค้อนที่มีรูตะแกรง 3 ขนาด (3.0 x 3.0, 3.0 x 2.5 และ 2.5 x 2.5 มิลลิเมตร) จากนั้นผ่านเครื่องเอ็กซ์ทรูดผลิตเป็นถั่วเหลืองไขมันเต็มเพื่อใช้เป็นวัตถุดิบที่ระดับ 25% ในอาหารสุกรหลังหย่านม การทดลองที่ 1 ศึกษาคุณลักษณะทางกายภาพ (ขนาดอนุภาคเฉลี่ย ค่าความเป็นเนื้อเดียวกัน ค่าพื้นที่ผิวสัมผัส ความสามารถในการไหลและความหนาแน่น) ต้นทุนการผลิต (กระแสไฟฟ้าที่ใช้และปริมาณผลผลิต) และสารต้านโภชนะ (ทริปซินอินฮิบิเตอร์ การละลายได้ของโปรตีน การเปลี่ยนแปลง pH และการเปลี่ยนแปลงสี) ของเมล็ดถั่วเหลือง ถั่วเหลืองบด ถั่วเหลืองไขมันเต็มและอาหาร การทดลองที่ 2 ศึกษาผลของขนาดอนุภาคถั่วเหลืองไขมันเต็ม ต่อคุณลักษณะการเจริญเติบโตและการย่อยได้ของสารอาหาร โดยใช้ลูกสุกรพันธุ์ผสม 3 สาย (H L D) คละเพศหย่านมที่อายุเฉลี่ย 21 $\pm$ 3 วัน จำนวน 36 ตัว เป็นเพศผู้ตอน 12 ตัวและเพศเมีย 24 ตัว สุกรแบ่งเป็น 3 กลุ่ม กลุ่มละ 4 ซ้ำ โดยใช้ซ้ำเป็นบล็อก ซ้ำละ 3 ตัว (เพศผู้ตอน 1 ตัว เพศเมีย 2 ตัว) ถูกเลี้ยงเป็นกลุ่ม ได้รับอาหารและน้ำอย่างเต็มที่เป็นเวลา 4 สัปดาห์ บันทึกน้ำหนักลูกสุกรและปริมาณอาหารที่กินทุกสัปดาห์ และ 7 วันก่อนสิ้นสุดสัปดาห์ที่ 2 และ 4 ของการทดลอง ผสมโครมิคออกไซด์ปริมาณ 4.0 ก./กก.อาหาร และเก็บมูลจากทวารหนักของลูกสุกรทุกตัวเพื่อวิเคราะห์การย่อยได้ของสารอาหาร

การทดลองที่ 1 พบว่าการลดขนาดรูตะแกรงของเครื่องบดแบบค้อนจาก 3.0 x 3.0 เป็น 3.0 x 2.5 และ 2.5 x 2.5 มิลลิเมตร ส่งผลให้ขนาดอนุภาคเฉลี่ยของถั่วเหลืองบด (P<0.001) ถั่วเหลืองไขมันเต็ม (P<0.05) และอาหาร (P<0.05) ลดลงอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ขนาดอนุภาคถั่วเหลืองไขมันเต็มที่ลดลง ส่งผลให้ความเป็นเนื้อเดียวกันสูงขึ้น (P<0.001) และพื้นที่ผิวสัมผัสเพิ่มขึ้น (P<0.05) แต่ไม่ส่งผลต่อความสามารถในการไหลและความหนาแน่น (P>0.05) ในขั้นตอนการบดนั้น การลดขนาดอนุภาคมีผลให้อัตราการผลิตลดลง (P<0.05) ขณะที่ความต้องการพลังงานเพิ่มขึ้น (P<0.001) อย่างไรก็ตามเมื่อคำนวณพลังงานไฟฟ้าและราคาต้นทุนการผลิตรวมทั้งการบดและการเอ็กซ์ทรูด พบว่าไม่มีความแตกต่างระหว่างขนาดอนุภาค (P>0.05) จากการวิเคราะห์สารต้านโภชนะทุกวิธีลดลงในทิศทางเดียวกันเมื่อได้รับความร้อนจากการบดและการเอ็กซ์ทรูด แต่ขนาดอนุภาคไม่มีผลต่อการลดลงของสารต้านโภชนะ การทดลองที่ 2 การลดขนาดอนุภาคถั่วเหลืองไขมันเต็มทำให้การย่อยได้ของวัตถุแห้งและโปรตีน(P<0.001) เพิ่มขึ้นที่ 2 สัปดาห์ของการทดลองแต่ไม่มีผลที่ 4 สัปดาห์ของการทดลอง ส่งผลให้ประสิทธิภาพการใช้อาหารเพิ่มขึ้น (P<0.05) ในสัปดาห์ที่ 2 และอัตราการเจริญเติบโตต่อวันเพิ่มขึ้น (P<0.01) ในสัปดาห์ที่ 2 และตลอดช่วงการทดลอง (P<0.05)

ภาควิชาสัตวบาล ลายมือชื่อนิสิต ชนิกานต์ ชิ้นปิ่นเกลียว

สาขาวิชาอาหารสัตว์ ลายมือชื่ออาจารย์ที่ปรึกษา

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CHANIKARN CHINPINKLEAW : EFFECTS OF FULL FAT SOYBEAN PARTICLE SIZES ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN POSTWEANING PIG. THESIS ADVISOR : ASSOC. PROF. SUWANNA KIJPARKORN, M.S., THESIS COADVISOR : ASST. PROF. NATCHANOK AMORNTHEWAPHAT, Ph.D., 55 pp.

Two experiments were studies to investigate the effects of particle sizes of full fat soybean on physical characteristic, production cost, anti-nutritional content, apparent fecal digestibility of nutrients and growth performance. Raw soybeans were ground with a hammer mill equipped with 3 sizes of splited screens (3.0 x 3.0, 3.0 x 2.5 and 2.5 x 2.5 mm opening area). Each ground soybean particle size was passed through the single-screw extruder to produce full fat soybean which was used as a feedstuff at the level of 25% in postweaning pig diet. In experiment 1, raw soybean, ground soybean, extruded soybean and diet were determined for physical characteristics (mean and standard deviation of particle size, surface area, angle of repose and bulk density) production cost (energy consumption and production rate) and anti-nutritional content (trypsin inhibitor, protein solubility, and urease index). In experiment 2, nutrient digestibility and growth performance were measured. Twelve barrows and twenty four female crossbred piglets (H L D) weaned at 21 $\pm$ 3 days of age were blocked by replicate allocated into 3 treatments which composed of 4 replicates of 3 pigs each (1 barrow and 2 females). The pigs were fed on their diets for a period of 4 weeks. Body weight and feed intake were weekly recorded. Seven days before the end of the 2nd and 4th week of experimental periods, chromic oxide were mixed (4 g/kg diet) and fed. Fecal samples were collected by rectal massage and analyzed for nutrients digestibility.

Experiment 1, reducing screens size of hammer mill from 3.0 x 3.0 to 3.0 x 2.5 and 2.5 x 2.5 mm. decreased mean of ground soybean particle size (P<0.001), full fat soybean particle size (P<0.05) and diet (P<0.05). The reducing of full fat soybean particle size increased uniformity (P<0.001) and surface area (P<0.05) but not differed for bulk density and angle of repose (P>0.05). Reducing particle size in grinding process decreased production rates (P<0.05) but increased energy consumption (P<0.001). However, no significant differences among particle size of total energy consumption and production cost (P>0.05) of both grinding and extrusion processes were found. All methods for analyzing anti-nutritional content, similarly decreased when heated in grinding and extrusion processes but particle size did not contribute to the decrease of anti-nutritional content. In experiment 2, reducing of full fat soybean particle size increased apparent DM and CP digestibility at 2nd week (P<0.001) but not at 4th week of experimental period and showed the positive impact on gain:feed ratio (P<0.05) at 2nd week and ADG (P<0.01) at 2nd week and the overall period (P<0.05) of the experiment.

Department Animal nutrition Student's signature Chanikarn Chinpinkleaw

Field of study Animal husbandry Advisor's signature S. Kijpark

Academic year 2007 Co-advisor's signature

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ABBREVIATION

ADFI	=	average daily feed intake
ADG	=	average daily gain
AOR	=	angle of repose
BAPA	=	benzoyl-DL-arginine-p-nitroanalide hydrochloride
cm	=	centrimetre
cm^2	=	square centrimetre
CP	=	crude protein
Cr_2O_3	=	chromic oxide
d	=	the diameter of the plate
d_{gw}	=	geometric mean particle size
d_i	=	diameter of i^{th} sieve in the stack
DM	=	dry matter
FCR	=	feed conversion ratio
FE	=	feed efficiency
FFSB	=	full fat soybean
g	=	gram
gain:feed	=	gain to feed ratio
GE	=	gross energy
h	=	height of the pile
Kcal	=	kilocalories
kg	=	kilogram
kWh	=	kilowatt-hour

L	=	litre
m	=	metre
m^2	=	square metre
mg	=	milligram
mg/g	=	mg pure trypsin inhibited/g of substrate
ml	=	milliliter
mm	=	millimetre
nm	=	nonometre
N	=	nitrogen
N	=	normality
rpm	=	round per minute
SA	=	surface area
S_{gw}	=	standard deviation of particle
t	=	ton
TIA	=	trypsin inhibitor activity
TIU/ml	=	trypsin inhibitor units per milliter
TNTC	=	too numerous to count
µm	=	micron
w_i	=	mass on i^{th} sieve
βs	=	shape factor for calculating surface area of particles
βv	=	shape factor for calculating volume of particles
ρ	=	specific weight of material

CHAPTER I

INTRODUCTION AND AIMS

The postweaning pig lag period limited digestive and absorptive capacity due to insufficient production of hydrochloric acid and pancreatic enzymes (Cramwell, 1995). The addition of antibiotics to feed could increase feed efficiency and growth in animals. Supplementation of antibiotics for extended periods of time, the pig's intestinal bacteria can become resistant to the antibiotics used. At slaughter, these resistant bacteria may enter the human food chain and cause illness in human (Cronwell, 2001). Thus, the use of antibiotic as a growth promoter was banded in many countries. At present, alternative feed additives or feed supplements such as enzyme, organic acids and herbs that have been considered to replace antibiotic. Feed processing technology is another alternative which can increase feed utilization and has been a focus of every feed company and producer in the swine industry. Research in this field was done for many years and found that decreasing particle size of grain in swine diets increases surface area, allowing for greater interaction with digestive enzymes and improved digestibility (Healy et al.,1994). Positive effects have been observed with reducing particle size of cereal grains for weaned pigs (Wu and Fuller, 1974; Healy et al., 1994), finishing pigs (Owsley et al., 1981; Giesemann et al., 1990; Wondra et al., 1995). It also improves the easy of handling and the mixing characteristics. However, fine grinding increase the energy costs of feed processing, the dust problems and the incidence of gastric ulcers in swine (Goodband et al., 2002).

Soybean is a major source of protein for animal diets. Liener (2000) reported that good growth could be demonstrated if soybeans were first heat treated before incorporation into the diet for animals. Later it was found that heating resulted in denaturation of anti-nutrient content that interfered with digestion. Full fat soybean is the whole seed after treatment to reduce anti-nutrient content. It has excellent amino acid composition and high level of lipid (Kohlmeier, 1996). There was limited information about the effects of soybean particle size. Therefore, the objectives of this study was to evaluate the effects of reducing particle sizes on production cost for FFSB processing, physical characteristic and anti-nutritional content of ground soybean, FFSB and diets. Furthermore, the effects of FFSB particle size on apparent fecal digestibility of nutrients and growth performance in postweaning pigs were also investigated.

CHAPTER II

BACKGROUND INFORMATION

2.1 Full Fat Soybean (FFSB)

Soybean is the oilseed crop that has been produced with the largest amounts in the world and is an important source of plant proteins in the diets of monogastric animals (Liener, 2000). Soybeans contain anti-nutritional factors to protect it from insect damage. Heat treatment during processing has been used to inactivate the anti-nutrient content in FFSB. FFSB is an excellent source of energy and protein, with special value in diets for young animals when a high nutrient concentration is required (Kohlmeier, 1996). The nutrient compositions of 100 g FFSB contain 10 g water, 38 g protein, 18 g fat, 5 g fiber, 4.1 g ash, 0.25 g calcium, 0.57 g phosphorus, 0.01 g sodium, 0.02 g chloride, 0.29 g magnesium, 55 mg vitamin E, 10 mg vitamin B1, 2.6 mg vitamin B2, 10 mg vitamin B6, 16 mg pantothenic acid, 23 mg niacin, 2000 mg choline, 3520 mg folic acid and 300 mg biotin (USDA, 1999).

2.2 Anti-nutritional factors of FFSB

Many feedstuffs contain anti-nutritional factors (Liener, 2000). These factors interfere with utilization of dietary nutrients that cause the inhibition of growth and feed efficiency that may affect the animal's health. Anti-nutritional factors can be classified in various ways. The following classification, based on their effect on the nutritive value of feedstuffs and the biological responses in animals (Huisman and Tolman,1992).

2.2.1 Lectins / hemaglutins :

Lectins are glycoproteins present in soybeans at a rate of 1 - 3% (Pieterse, 2000). Glycoproteins are varies in molecular weight and in chemical structure and one of their characteristic is to bind with specific sugars (Liener, 2000). Lectins have an ability to bind to carbohydrate-containing molecules on the epithelial cells of the intestinal mucosa, in which the extent of this binding determines its toxicity (Lewis and Southern, 2001). This leads to growth reduction as a result of the negative effects of the anti-nutritional factors (Liener, 2000). Lectins are quite unstable in heat therefore heat processing appears to be an effective means to inactivate lectins (Calvalho and Sgabieri, 1997). Level of lectin dropped approximately 100-fold when the raw soybean was processed into defatted or toasted soybean meal (Padgette et al., 1996). Fasina et al. (2003) reported that steam heating soybean meal at 100 $^{\circ}$C for 5 to 10 minutes were sufficient to inactivate lectin activity.

2.2.2 Protease inhibitors :

The main protease inhibitors are the trypsin and chymotrypsin inhibitors. These are peptides which form stable inactive complexes with some of the pancreatic enzymes. As a result the activities of both trypsin and chymotrypsin are reduced (Huisman and Tolman, 1992). This reduces protein digestibility and causes pancreatic swelling as this gland attempts to produce more enzymes to make up for the excreted loss. The lost enzymes, being rich in sulphur amino acids, further reduce the amino acid status of the animal (Liener, 2000). Trypsin and chymotrypsin inhibitors are also heat sensitive and their activities in raw beans may be reduced to insignificant levels by adequate heat processing making it safe to include in diets for non-ruminants (Pieterse, 2000).

2.2.3 Saponins :

Saponins are steroid or triterpenoid glycosides that are present in many feedstuffs. They can form foam in aqueous solutions and haemolyse red blood cells (Liener, 2000). The effect of saponins is to slow down growth performance in pigs (Cheeke and Shull, 1985). Johnson et al. (1986) reported that saponins from several plant species also rapidly increase the permeability of small intestine in vitro, leading to the increased uptake of poorly permeable substances and a loss of normal function. In general saponins are of minor concern in monogastric animals because saponins are present in very low levels in feedstuffs (Huisman and Tolman, 1992).

2.2.4 Phytic acid :

Phytic acid is called the hexaphospho-myoinositol. Liener (2000) estimates that two-thirds of the phosphorus in soybeans is bound as phytate and unavailable to animals. This compound chelates mineral nutrients including calcium, magnesium, potassium, iron, and zinc, rendering them unavailable to monogastric animals consuming the beans (NRC, 1998). The charged phosphate groups on phytic acid can form electrostatic associations with the terminal amino group on proteins or with the free amino group on lysine and arginine residues within protein molecules. In addition, phytate–mineral–protein complexes can form with multivalent cations acting as a bridge between phosphate groups on the phytate molecule and the terminal carboxyl group of proteins or free carboxyl group on aspartate and glutamate residues within protein molecules. Phytate binding to proteins and minerals in the digesta has the potential to impair the activity of digestive enzymes (Cheryan, 1980). Phytic acid naturally occurs in soybeans and most soybean products and can make up to 1 - 1.5% of the dry weight. Heat treatment has been also shown to reduce levels of phytic acid (Liener, 2000).

2.2.5 Urease :

Urease is an enzyme that has the ability to convert urea to ammonia. When unprocessed soybeans were mixed with urea, then ammonia will be release. Raw soybeans have a variable urease activity and it has not much of a nutritional implication other than as an indirect assessment of the degree of adequacy of processing (Mateos et al., 2001). Urease enzyme can be inactivated by heat treatment of soybean and its inactivation rate is similar to that of trypsin inhibitors (Osella et al., 1997). Hence, urease activity is practically used as a criterion for quality control of soybean meal.

2.3 Heat treatment

Due to the presence of anti-nutritional factors, the nutritional value of raw unprocessed soybean is relatively low. Heat treatment has been used to inactivate the heat labile anti-nutritional factors present in raw soybeans. The processing conditions (time, temperature, moisture and particle size) have an effect on anti-nutritional factors and nutritive value of the diet. The three best known used to treat soybeans are cooking, roasting and extrusion.

2.3.1 Cooking :

This is a relatively simple process to use. The raw soybeans are immersed in water and cooked for between 30 and 120 minutes. This is the more traditional method used by local soybean producers (Mateos et al., 2001).

2.3.2 Roasting :

The heat used for roasting can be generated by an oven, a coal burner or directly by a flame. The temperature reached varies between 110 and 170 °C depending on the equipment used (Katic et al., 1996). It is recommended that the exit temperature of the soybeans should be between 110 and 113 °C for monogastics. This process reduces the initial moisture of the soybean by 30% but without breaking down the cellular structures (Thomason, 1987).

2.3.3 Extrusion :

About 80% of cooking soybean using in feed mill industry was produced by extrusion method. In this process, whole soybeans receive the heat not only from the barrel wall, but also from the friction forces between screw and barrel while being passed through the barrel. The extruder is operated in a continuous manner at high temperature for a relatively short time. The heat generated from the friction and shearing forces in the extruder barrel typically raised the temperature to 104 – 115 °C at a feed rate of 1 ton/hour, corresponding to a retention time of 2.9 minutes (Wiriyaumpaiwong et al., 2004). The extrusion is a highly effective process in terms of enhancing the energy value of the soybeans for monogastrics as it breaks down the cellular structure of the cotyledons and therefore releases almost all of the fat contained within the spherosomes, making it more accessible for the animal (Mateos et al., 2001).

2.4 The degree of adequacy of heat on processing method

The challenge in soybean processing is to apply the optimum amount of heat to produce the most nutritious product. Insufficient heating, or under processing, will negatively impact amino acid digestibility because the anti-nutritional factors are not adequately destroyed. Excessive heating, or over processing, will negatively impact amino acid digestibility because a portion of the amino acids have either been destroyed or tied-up as indigestible, bound compounds (Dudley-Cash, 1999). The degree of adequacy of heat on processing method should be determined as follows below.

2.4.1 Urease index :

The principle of the urease index method is that the urease enzyme is present in soybean. The urease index is an indirect indicator of the present of anti-nutritional factors, such as trypsin inhibitors (Kohlmeier, 1996). On the basis of the assumption that the urease enzyme in raw soybeans is denatured at approximately the same rate as the trypsin inhibitor, and because it is easier to determine urease activity than trypsin inhibitor, urease assays have generally been used by the feed industry in monitoring soybean meal quality (Casket and Knapp, 1944). The urease assay is based on pH increase from ammonia which is released from urea by residual urease enzyme in soybean meal. The destruction of the urease enzyme in soybean meal by heating is correlated to the destruction of trypsin inhibitors and lectins. The optimum increased pH has generally been considered to be 0.05 – 0.20 (Table 2.1) (Garlich, 1988; Dudley-Cash, 1999) and 0.05 – 0.15 (AACC, 1990). In addition, the simple method for feed manufacturer for judgement of soybean processing quality to determined by changing of color. There was limited information in color change for soybean heated. McNaughton et al. (1981) reported that the *L* value (lightness) was decreased from 70.05 to 50.33 and *a* value (redness) was increased from 3.21 to 7.09 when soybean meal autoclaved from 0 to 25 minutes. The change in *L* and *a* value can predict trypsin inhibitor content and over-processing of soybean meal because the formation of brown pigments as a result of Maillard reaction continued even though all trypsin inhibitor has been destroyed. They suggested that *a* value should be 4.5 - 5.5 and pH change less than 0.15 to indicate processing adequacy.

<u>Table 2.1</u> Urease index values of processed soybeans

Conditions	Urease index (Δ pH)
Raw soybean	2.00
Under processed	> 0.20
Well processed	0.05 – 0.20
Over processed	< 0.05

Garlich, 1988

2.4.2 Protein solubility :

Protein solubility is also an important parameter that characterizes the protein quality of soybean. The protein solubility was reduced after heat treatment. The disulfide bond of proteins which links between amino acid groups was destroyed therefore the proton of hydrogen in water molecules inside proteins reacted with the sulfur of disulfide to form as sulfhydryl and protein molecules aggregated (Prachayawarakorn et al., 2004). It is suggested that the protein solubility index should not below 70 to 75% in commercial soybean meal (Dudley-Cash, 1999).

2.4.3 Trypsin inhibitor content :

Trypsin inhibitor levels are closely paralleled by urease content and as urease content is easier to determine, it is commonly used as an indicator of anti-nutritional factor destruction and thus to predict processing adequacy, especially under-processing. Zarkadas and Wiseman (2005) reported that trypsin inhibitor of soybean ranged from 24.1, 16.8, 6.4 and 2.9 mg pure trypsin inhibited/g of substrate with increasing extrusion temperature to 70, 90, 110 and 150 $^{\circ}$C respectively. The best performance results were obtained when trypsin inhibitor levels in soybean lower than 5 mg/g. This value was only obtained when the extruder temperatures was greater than 110 $^{\circ}$C (Chang et al., 1984).

2.5 Particle size

Most of the feed ingredients used in feed manufacturing are subjected to particle size reduction either within the feed plant or prior to receiving. Crenshaw (2001) reported that the greatest benefits from particle size reduction in feed manufacturing processes are related to:

1) Improves feed conversion ratio
2) Improves uniformity of mixed feed
3) Reduces amount of segregation of delivered feed

4) Improves digestibility of nutrients by increasing surface area

Classification of determining particle size has been developed based on the geometric diameter of particles measured in microns and the geometric mean standard deviation of the particles or their distribution. Goodband et al. (2002) classified particle size by diameter into 4 ranges (Table 2.2).

Table 2.2 Description of particle size distribution in feed ingredients

Diameter (μm)	Coarse	Medium	Fine
2,000 – 4,000	36%	17%	0%
1,000 – 2,000	52%	64%	14%
500 – 1,000	6%	13%	53%
< 500	6%	6%	33%

Goodband et al., 2002

2.6 Feed milling equipment

Quality swine feed can be manufactured on the farm with many different types of equipment however the mill operator must fully understand the limitations of the feed processing equipment. Common methods of on-farm particle size reduction involve two types of mills (hammer mills and roller mills).

2.6.1 Hammer mills :

Hammer mills reduce particle size by 1) explosion from the impact of the hammers; 2) cutting by the edge of the hammers and screen; and 3) rubbing action or attrition. Advantages of hammer mills are the ability to handle any combination of grains and low maintenance costs. The size of the openings in the hammer mill screen greatly determines the size of particles which are produced (Crenshaw, 2001). It is difficult to make a specific recommendation for one screen for each type of grain; however, screen size should be adjusted to produce a mean particle size of 700 µm. In addition, there are several factors, which may be change to particle size such as hammer mill revolutions per minute, tip speed and moisture content of grain (Goodband et al., 2002).

2.6.2 Roller mills :

Roller mills have the advantage of creating a more uniform particle size than a hammer mill. Roller mills normally have a higher initial purchase cost but are less expensive to operate. Particle size may be controlled in a roller mill by the setting of the rollers, corrugations, spiral roller versus non-spiral and speed differential of the rollers. Roller mills are limited to non-fibrous products. Most manufacturers will also recommend a differential drive of 10 to 25 percent with rolls turned so that the sharp edge of each roll meets the grain. Roll speed ranges from 350 to 600 rpm depending upon surface speed. This low speed will create less dust and wear (Crenshaw, 2001).

2.7 Particle size on energy cost

Wondra et al. (1995) reported that the energy consumption was increased 40.7% and production rate was decreased 3.7% as the particle size of corn was reduced from 1,000 to 600 µm. However, the effects of grinding size vary according to type of cereal. Similar result was reported by Reece et al. (1986) that the energy consumption was increased 35% and production rate was decreased 27% as grinding corn with a hammer mill screen size increased from 4.76 to 6.35 mm. The energy consumption and production rates of reducing corn particles size were shown in Figure 2.1.

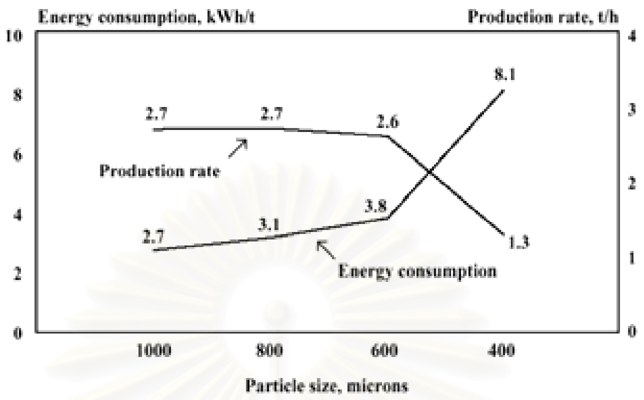

Figure 2.1: Energy consumption and production rates when hammer milling corn (Dritz and Hancock, 2001)

2.8 Particle size on physical characteristics

Feed manufacturers are generally only interested in the mean particle size (d_{gw}), standard deviation (S_{gw}), surface area and bulk density. The recommended d_{gw} for swine diets is 600 to 800 µm (Goodband et al., 2002). The S_{gw} is a measurement of the particle size variation about the average or uniformity particle size. Most feed samples will have S_{gw} a ranging from 2.0 to 2.4. The best S_{gw} is 1.0 (Baker and Herrman, 2002). Surface area of grains and diet increased as particle size was decreased (Healy et al., 1994). Wang et al. (1995) showed that fine soybean meal particle sizes had a higher bulk density than coarse particles. The flow ability of the diet is measured by the angle of repose (AOR), which is defined as the maximal degree at which a pile of material retains its slope (Apple, 1994). Lawrence et al. (2003) reported that reducing particle size of extruded expelled soybean meal or solvent extracted soybean meal increased the angle of repose.

2.9 Particle size on nutrient digestibility

Smaller particle size of cereal grains is thought to provide greater surface area for steam conditioning (MacBain, 1966) and improves digestibility of nutrients, which allows digestive enzymes in the pig's digestive system to digest the nutrients in the feed. Digestibility of protein, energy and other nutrients is generally improved so that affect on feed conversion ratio (FCR) improved (Giesmann et al., 1990). Wondra et al. (1995) reported that increased digestibility of dry matter (DM), nitrogen (N) and gross energy (GE) with increased particle uniformity. In addition, GE digestibility increased as particle size of corn was reduced from 1,000 to 400 µm (P<0.05). Similar results were reported in experiments by Fastinger and Mahan (2003) studied the effect of particle size reduction of solvent-extracted soybean meal on amino acid digestibility in growing pigs, the results showed that particle size reduction to 185 µm increased 1.4% apparent digestibility and 1.3% true digestibility of some amino acids, particularly the essential amino acids isoleucine, leucine, methionine, phynylalanine and valine. The largest increase in digestibility was evident when the particle size was reduced from 900 to 600 µm. Supported by Owsley et al. (1981) who reported that reducing the particle size of sorghum from 1,200 to 802 µm in grower-finisher diets resulted in a 7.6% increase in apparent amino acid digestibility.

3.0 Particle size on growth performance

Nutrients digestibility affected on growth performance of animal. Healy et al. (1994) evaluated growth performance of pigs weaned at 21 days of age and fed starter diets in which the grain (corn and hard or soft endosperm) was ground to 900, 700, 500, or 300 µm. For 0 to 14 days after weaned, decreasing in average daily gain (ADG) (*P*<0.009) and gain:feed ratio (*P*<0.002) as particle size was reduced from 900 to 300 µm. However, average daily feed intake (ADFI) was not affected by particle size. For 14 to 35 days after weaned, decreasing in ADG and ADFI as particle size was reduced from 900 to 300 µm (*P*<0.04). They suggested that response of reducing particle size was greatest during the first 2 weeks of postweaning and the optimum particle size between 500 and 700 µm. Wu and Fuller (1974) found that improved ADG and FCR for the weaning pigs (28 to 35 days of age) when the screen size of hammer mill used for maize grinding was decreased from 9.5 to 1.6 mm. In contrast with Lawrence et al. (2003), they showed that reducing of extruded-expelled soybean meal particle size from 965 to 639 µm and solvent-extracted soybean meal particle size from 1,266 to 444 µm at the inclusion rate 34.4% diet resulted in no differences in ADG, ADFI and gain:feed ratio of weaned pigs (35 days of age). They also reported that the changing in soybean meal particle size had a relatively minor impact on entire diet particle size due to its inclusion rate compared to corn. For finishing pigs, experiment by Wondra et al. (1995) showed that decreased ADFI (*P*<0.002) and increased FE (*P*<0.001) as corn particle size was decreased from 1,000 to 400 µm. Similar results were reported by Mavromichalis et al. (2000) when particle sizes of wheat was decreased from 600 to 400 µm.

Negative effects have been observed with reducing particle size of cereal grains for pigs such as increased respiratory problem from higher dust levels, increased costs of feed processing (Crenshaw, 2001) and increased incidence of gastric ulcers. The later cause by finely ground is more fluid when mixed with the digestive secretions compared to coarsely ground feed. As a result, the acids in the stomach have a greater chance of coming into contact with and irritating the esophageal region of the stomach. The frequency of ulceration increases when particle size drops below 500 μm (Goodband et al., 2002).

CHAPTER III

MATERIALS AND METHODS

Two experiments were conducted to determine the following objectives.

1) the effects of reducing soybean particle sizes on production cost of grinding and extrusion processes
2) the effects of particle sizes of ground soybean, FFSB and diets on physical characteristic and anti-nutritional content
3) the effects of FFSB particle sizes on apparent fecal digestibility of nutrients and growth performance in postweaning pigs

Experiment 1

Preparation of FFSB

Raw soybean was ground with a hammer mill (4 rows of 60 hammers, speed 1,400 rpm) (Buhler, Switzerland) equipped with split screens of 3.0 x 3.0, 3.0 x 2.5 and 2.5 x 2.5 mm opening area for the control group, the second and third group respectively. Each ground soybean particle size was extruded through the single-screw extruder (TEX6000F, Triumph, Thailand) with a barrel length of 2.6 m., 1 die opening area and a die diameter of 0.02 m. The set up of barrel temperatures were 65, 115, 100 and 100 $^{\circ}$C for section 1 to 4 respectively.

Sample and data collection

Grinding and extrusion processes were continuous process with constant condition according to the manufactory, only required screen of hammer mill was changed. Data and Sample from both processes were collected every 20 minutes-interval after start up at 100 minutes. Thus, there were five samples collection for each FFSB particle size.

1) Data collection;

Both grinding and extrusion processes, production rate was determined from the total weight of soybean produced and the production time. The energy consumption was recorded via monitor screen and the production time for each particle size was measured with a digital clock to calculate the electrical energy consumption. Electrical energy charge rates were based on the price of 2.695 baht/unit. Feeder rate was also recorded via monitor. In addition for extrusion process, four sections temperature of extruded barrel were immediately recorded with an insulated container equipped with a probe thermometer (Primus, Thailand). Five times of all parameters were collected for data analysis.

2) Sample collection;

Raw soybean, ground soybean and FFSB were collected approximately 300 grams (2 plastic bags) per time and kept in freezer (-20 °C) for physical characteristics, nutritional contents and anti-nutritional content analysis. For physical characteristics, five samples per treatment were random for analysis.

2.1 Physical characteristics were analyzed by the methods as follow:

2.1.1 Particle size distribution

Particle size distribution was analyzed by the methods of ASAE (1983). The equipments required for particle size analysis were a scale, shaker and sieves set with diameter opening 1191, 841, 594, 420, 297, 212, and 150 µm. The procedure in brief: put one hundred gram of each samples on the top sieve and place sieve stack on the shaker for 10 minutes. Remove the sieves stack from shaker and weigh each sieve with the retained material and record. After that clean the sieve and weigh back the empty sieve and record. Weight of sample on each sieve diameter was calculated by minus empty sieve from sieve with sample.

The geometric mean particle size (d_{gw}) can be calculated as follows:

$$d_{gw} = \log^{-1}\left\{\frac{\sum (w_i \log d_i)}{\sum w_i}\right\}$$

w_i = mass on i^{th} sieve (g)

d_i = diameter of i^{th} sieve in the stack

The standard deviation (S_{gw}) can be calculated as follows:

$$S_{gw} = \log^{-1}\left\{\frac{\sum (w_i (\log d_i - \log d_{gw})^2}{\sum w_i}\right\}^{0.5}$$

The surface area (SA) can be calculated as follows:

$$SA = \frac{\beta s}{\rho \beta v} \exp(0.5 \ln^2 S_{gw} - \ln d_{gw})$$

βs = shape factor for calculating surface area of particles

βv = shape factor for calculating volume of particles

ρ = specific weight of material

2.1.2 The angle of repose (AOR)

The angle of repose of the sample was measured by filling an 6-cm-diameter PVC cylinder with 150 g of the material to be tested. Then, the cylinder was raised and the material will fall away, leaving a cone-shaped pile on the 6-cm-diameter plate. The angle of repose, or maximal angle at which a pile of material retains its slope, could be determined by taking the inverse tangent of the pile height (h) divided by one half the diameter (d) of the plate (Adapt from Apple, 1994).

$$AOR = \tan^{-1} \frac{2h}{d}$$

h = height of the pile

d = the diameter of the plate

2.1.3 Bulk density

Bulk density was measured by filling a 250-ml glass cylinder with 100 g of sample then the scale of cylinder was recorded (Chung and Converse, 1971).

2.2 Nutritional content was analyzed using proximate analysis (AOAC, 1990)

2.3 Anti-nutritional content was analyzed by the methods as followed:

2.3.1 Trypsin inhibitor

Trypsin inhibitor activity (TIA) was analyzed by AOCS Official method (1997). TIA was measured by the loss of activity of added trypsin under standard conditions. Weigh 1 g of defatted sample into a suitable beaker containing a magnetic stirring bar. Add 50 ml of 0.01 N sodium hydroxide solution. Slowly agitate suspension 3 hours at room temperature. At the end of 3 hours, the pH was determined. The value should be in between 8.4 and 10.0. With serological pipet, add 0, 0.6, 1.0, 1.4 and 1.8 ml of the diluted suspension to duplicate sets of test tubes. Then, add sufficient water to bring the volume in each test tube to 2.0 ml and add 2 ml of trypsin solution (4 mg trypsin in 200 ml of 0.001 N hydrochloric acid) and place in a water bath at 37 $^{\circ}$C. After exactly 10 minutes, add 5 ml of benzoyl-DL-arginine-p-nitroanalide hydrochloride (BAPA) solution (40 mg BAPA in 1 ml dimethyl sulfoxide and dilute to 100 ml with hydroxymethyl aminomethane buffer) in each test tube and stop the reaction in each tube by adding 1 ml of 30% acetic acid solution after a second 10 minutes incubation phase. Determine the absorbance of each test tube by UV-VIS spectrophotometer (Shimadzu® UV 1201) at 410 nm.

Trypsin inhibitor units per milliliters diluted sample (TIU/ml) can be calculated as follows:

$$\text{TIU/ml} = \frac{100 \times (\text{absorbance of blank} - \text{absorbance of sample})}{\text{Number of ml of diluted sample suspension}}$$

Milligram pure trypsin inhibited per gram of substrate (mg/g) can be calculated as follows:

$$\text{mg/g} = \frac{\text{TIU}}{\text{ml}} \times \text{dilution factor} \times 0.05$$

2.3.2 Protein solubility

Protein solubility was determined by the procedure described by Araba and Dale (1990). In brief, approximately 1.5 g of sample was added in 0.2% potassium hydroxide solution and placed in 22 $^{\circ}$C water bath for 20 minutes. Solution was centrifugation (HERMLE Z400K, New Jersey, USA) at 1,250 rpm for 10 minutes. The nitrogen content of the supernatant was determined by Kjeldahl method (AOAC, 1990).

2.3.3 Urease index

2.3.3.1 pH change

Approximately 0.2 g of sample was added in 10 ml of buffered urea solution (15 g urea and 500 ml phosphate buffer solution were mixed and adjusted pH 7.0) and placed in 30 $^{\circ}$C water bath for 30 min. pH was determined using pH meter (Metler Toledo Delta 340, USA) with combination electrode. Record different in terms of pH between test and blank runs as urease index (AACC, 1990)

2.3.3.2 Color Value

Approximately 50 g of sample was poured in to a glass petri dish and added with 50 ml phenol red solution (1.4 g phenol red in 70 ml of

0.1 N sodium hydroxide and 3 L of urea solution were mixed) for reaction. After 5 minutes, spots of red color were immediately counted and determined by Hunter Miniscan XE (Adapt from Juttupornpong, 1996). The mean of five sections data (center and four section diagonal on petri dish) were reported. The redness, greenness, yellowness and blueness represented by *+a, –a, +b* and *–b* respectively. The *L* value gives a spectrum in the range from 0 to 100 with the highest value showing whiteness whereas the lowest value indicates the blackness (Prachayawarakorn et al., 2004).

Experiment 2

This study was approved by Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University.

Animal and management

Twelve barrow and twenty four female weaned crossbred piglets (Hampshire x Lanrace x Duroc), age 21 $\pm$ 3 days were used. Following an adaptation period of 3 days, all animals were blocked by replication allocated into 3 treatment diets which composed of 4 replications of 3 pigs each. Each replication was composed of 1 barrow and 2 female piglets. Pigs were raised in pen size 4.25 m^2 which provided with four-hole self-feeder and one nipple drinker per pen for 4 weeks. All piglets received diets and water *ad libitum* through the experimental period.

Feed and feeding

All diets containing FFSB from the experiment 1 at the level 25% of diet; other ingredients and calculated analysis of nutritional content are shown in table 3.1. Diets were calculated according to NRC requirement (NRC, 1998). Experimental diets were collected and kept in freezer at -20 °C until analysis. Gross energy of feed was analyzed using bomb calorimeter (Parr 1241, Illinois, USA).

During days 11 to 17 and days 25 to 31 of the experimental period, chromic oxide was added to all diets at the level of 4 g/kg feed as an indigestible dietary marker.

Table 3.1 The ingredient composition and calculated analysis of the experimental diets.

Ingredients	Amount (kg/100kg diet)
Broken rice	36.62
Full fat soybean	25.00
Corn	20.00
Soybean meal	5.00
Fish meal, 61%CP	5.00
Whey	5.00
Limestone	0.41
Mono-dicalcium phosphate, 21%CP	0.65
Salt	0.16
Choline chloride	0.10
DL-Methionine	0.50
L-Lysine	0.10
L-Tryptophan	0.07
L-Threonine	1.29
Premix[1]	0.70
Calculated analysis (%)	
Protein	22.30
Fat	6.92
Fiber	3.48
Calcium	0.75
Total phosphorous	0.63
Methionine	0.40
Lysine	1.32
Tryptophan	0.34
Threonine	1.46
Salt	0.35
ME, Kcal/Kg	3285.00

[1] Premix per kilogram of diet provided: 12,000 IU of vitamin A, 2,400 IU of vitamin D3, 18 mg of vitamin E, 3 mg of vitamin K, 1.2 mg of vitamin B1, 3.6 mg of vitamin B2, 1.8 mg of vitamin B6, 0.018 mg of vitamin B12, 16 mg of pantothenic acid, 0.6 mg of folic acid, 0.1 mg of biotin, 300 mg of choline,42 mg of Mn, 120 mg of Zn, 100 mg of Fe, 1500 mg of Cu, 1.5 mg of I, 0.84 mg of Co and 0.2 mg of Se.

Data Collection

The temperature and relative humidity were daily recorded at 08:00 am, 12:00 and 17:00 pm. Health status and mortality were recorded on daily basis. Body weight and feed intake were weekly recorded and used to calculate growth performance. The formula is shown below.

$$\text{Average daily gain (ADG, g/day)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Days}}$$

$$\text{Average daily feed intake (ADFI, g/day)} = \frac{\text{Feed intake}}{\text{Days}}$$

$$\text{Feed efficiency (FE)} = \frac{\text{Average daily gain}}{\text{Average daily feed intake}}$$

Sample Collection

On days 15 to 17 and 29 to 31 of the experiment, fecal samples were collected from all pigs by rectal massage (Healy et al., 1994) (at 10:00 and 15:00). Samples in each pen were equally pooled and dried to constant weight at 60 °C in the hot air oven. The dried samples were kept in freezer (-20 °C) until analysis for nutritional composition by AOAC (1990). Chromic oxide concentration in diet and feces were analyzed by Bolin et al. (1952). In brief, approximately 1.5 g of diet or 0.5 g of feces, was mixed with 12 ml of oxidizing reagent (11.75 g sodium molybdate, 150 ml of 96% sulfuric acid and 200 ml of perchloric acid were mixed) for first digestion phase. After 1 hour, 3 ml perchloric acid was added and followed by a second digestion phase. Then, solution was adjusted to 100 ml by distilled water. The absorbance of the solutions was measured by UV-VIS spectrophotometer (Shimadzu® UV 1201) at 410 nm.

The percentage of apparent fecal digestibility can be calculated as follows:

$$\text{\% apparent fecal digestibility} = 100 \times \left\{ 1 - \frac{\%Cr_2O_3 \text{ in diet} \times \%\text{Nutrient in feces}}{\%Cr_2O_3 \text{ in feces} \times \%\text{Nutrient in diet}} \right\}$$

Cr_2O_3 = chromic oxide

Statistic analysis

Processing and physical characteristics data were analyzed as complete randomized design. All growth performance and nutrient digestibility data were analyzed as a randomized complete block designs, replications were defined as the block factors. The GLM procedure of SAS (2003) was used to determine the main effect of 3 particle sizes of FFSB. Treatment comparisons were made using Duncan's New Multiple Range Test (Steel and Torrie, 1962). The level of significant difference was set at $P<0.05$.

CHAPTER IV

RESULTS

<u>Experiment 1</u>

Physical characteristics

Particle size (d_{gw}) of ground soybean after passed through the splited screens size 3.0 x 3.0, 3.0 x 2.5 and 2.5 x 2.5 mm of hammer mill were 428, 351 and 307 µm respectively. The d_{gw} of FFSB after grinding and extrusion processes were 945, 879 and 822 µm respectively. Finally, diet particle sizes were 778, 705 and 665 µm respectively. Both d_{gw} of ground soybean and FFSB showed significant differences ($P<0.001$ and $P<0.05$ respectively) between the screen sizes 3.0 x 3.0 and 3.0 x 2.5 or 2.5 x 2.5 mm. Reducing particle size decreased average standard deviation of particle (S_{gw}) but increased surface area. No significant differences of bulk density and the angle of repose were found when particle size decreased except angle of repose in ground soybean ($P<0.05$) (Table 4.1).

<u>Table 4.1</u> The characteristics [1] of ground soybean, full fat soybean (FFSB) and diet from different splited screen size of hammer mill

Items	Hammer mill screen size, mm			SE	*P*-value
	3.0 x 3.0	3.0 x 2.5	2.5 x 2.5		
Ground soybean [2]					
d_{gw}, µm [3]	428^{A}	351^{B}	307^{C}	7	<0.001
S_{gw} [4]	1.77^{A}	1.71^{B}	1.68^{B}	0.02	<0.001
Surface area, cm^2/g	127^{C}	147^{B}	167^{A}	3	<0.001
Angle of repose, °	55.0^{C}	59.2^{B}	65.5^{A}	0.8	<0.001
Bulk density, g/L	491	495	495	2	0.101
FFSB [2]					
d_{gw}, µm [3]	945^{a}	879^{ab}	822^{b}	26	0.006
S_{gw} [4]	1.97^{A}	1.93^{AB}	1.88^{B}	0.01	<0.001
Surface area, cm^2/g	60.0^{b}	64.3^{ab}	66.9^{a}	0.9	0.007
Angle of repose, °	48.3	50.1	53.0	1.4	0.059
Bulk density, g/L	526	529	531	2	0.252
Diet [2]					
d_{gw}, µm [3]	778^{a}	705^{ab}	665^{b}	22	0.026
S_{gw} [4]	2.18	2.18	2.14	0.07	0.932
Surface area, cm^2/g	78.0^{b}	85.7^{ab}	90.1^{a}	2.4	0.030
Angle of repose, °	51.0	53.6	55.4	1.7	0.379
Bulk density, g/L	649	656	661	4	0.136

[1] 15, 15 and 3 observations per means for ground soybean, FFSB and diet respectively

[2] Moisture content of ground soybean 10.05, 10.05 and 10.07%; FFSB 10.16, 10.15 and 10.17%; diets 11.26, 11.17 and 11.20% for screen sizes 3.0 x 3.0, 3.0 x 2.5 and 2.5 x 2.5 mm of hammer mill respectively.

[3] Average geometric means diameter particle size (ASAE, 1983).

[4] Average standard deviation of particles (ASAE, 1983).

[A, B, C] Means within the same row with different superscripts differ highly significant (P<0.001).

[a, b] Means within the same row with different superscripts differ significantly (P<0.05).

Production cost

In the grinding process, production rate decreased from 2.55 to 1.73 tons/hour (P<0.05) while energy consumption increased from 2.78 to 5.49 kilowatt hours/ton (P<0.001) when decrease screens size of hammer mill from 3.0 x 3.0 to 2.5 x 2.5 mm. However, no significant differences of energy consumptions were found when decrease screens size of hammer mill from 3.0 x 3.0 to 3.0 x 2.5 and 2.5 x 2.5 mm.

In the extrusion process, production rate, energy consumption and steam pressure were not changed but feeder rate increased from 40.2 to 40.4 round per minute (P<0.05) when particle size decreased. Steam pressure fed to the pre-conditional was constant, thus temperature in pre-conditional and in each section of extruder barrel were not significantly changed. The pre-conditioner temperatures were between 81.7 – 83.7 $^{\circ}$C and temperature within an extruder barrels were the lowest in 1st section and highest in 2nd section. Total energy consumption and calculated production cost of both grinding and extrusion processes showed no significant differences among particle sizes (Table 4.2).

Table 4.2 Effect of particle size on grinding and extrusion processes[1]

Items	Hammer mill screen size, mm			SE	*P*-value
	3.0 x 3.0	3.0 x 2.5	2.5 x 2.5		
Grinding : Particle size , µm	428	351	307		
Energy consumption, kWh/t	2.78^{B}	5.06^{A}	5.49^{A}	0.22	<0.001
Production rate, t/h	2.55^{a}	2.17^{ab}	1.73^{b}	0.16	0.019
Extrusion : Particle size, µm	945	879	822		
Energy consumption, kWh/t	9.27	9.38	9.48	0.62	0.971
Production rate, t/h	7.36	7.35	7.33	0.11	0.986
Feeder rate, rpm	40.2^{b}	40.4^{a}	40.4^{a}	0.1	0.019
Steam Pressure, bar	3.50	3.50	3.50	0.00	0.000
Pre-conditioner Temperature, °C	81.7	83.3	83.7	0.9	0.233
Extrusion Temperature, °C					
Section 1	65.4	68.6	66.4	1.1	0.142
Section 2	118	117	115	2	0.387
Section 3	108	109	109	1	0.818
Section 4	97	100	100	1	0.066
Grinding and Extrusion processes					
Total energy consumption, kWh/t	12.0	14.4	14.9	0.72	0.057
Production cost, baht/t[2]	35.2	41.7	41.8	1.95	0.057

[1] 5 observations per means

[2] Electrical energy charge rate based on the price of 2.695 baht/unit.

[A, B] Means within the same row with different superscripts differ highly significant (P<0.001).

[a, b] Means within the same row with different superscripts differ significantly (P<0.05).

Nutritional content of soybean and diets

The chemical analysis of all nutrients show a little changed in ground soybean, FFSB and diet in each particle sizes after passed grinding process, extrusion process and mixing with other ingredients respectively (Table 4.3).

Soybean and diets quality

There was a little changed in the urease index (pH change and color value), protein solubility and trypsin inhibitor activity of ground soybean, FFSB and diet among particle sizes. The pH change of raw soybean fell rapidly from 2.14 units to near zero after passed extrusion process and mixed with other ingredients. For color value, the *L* value and *b* value increased but *a* value and red colored spots decreased when soybeans were incrementally heated (Table 4.4).

Table 4.3 Chemical analysis of nutritional content of soybean and diets (dry matter basis)

Items	Raw soybean[1]	Ground soybean particle sizes[2], μm			FFSB particle sizes[3], μm			Diet particle sizes[4], μm		
		428	351	307	945	879	822	778	705	665
Crude protein, %	39.3	38.1	39.1	38.6	37.6	37.8	36.7	21.2	21.2	21.2
Ether extract, %	19.5	20.3	20.4	20.4	20.7	20.7	20.7	6.68	6.66	6.74
Crude fiber, %	4.75	4.76	4.73	4.73	4.99	4.95	4.98	3.48	3.44	3.39
Ash, %	6.50	6.54	6.54	6.52	6.53	6.52	6.50	6.00	5.96	5.99
Calcium, %	0.30	0.29	0.30	0.30	0.30	0.29	0.28	0.82	0.82	0.87
Total phosphorus, %	0.63	0.66	0.68	0.64	0.68	0.68	0.67	0.78	0.80	0.77
Gross energy, Kcal/Kg	-	-	-	-	-	-	-	3682	3693	3660

[1] Moisture content of raw soybean 10.04%

[2] Moisture contents of ground soybeans 10.05, 10.05 and 10.07% for screens size 3.0 x 3.0, 3.0 x 2.5 and 2.5 x 2.5 mm of hammer mill respectively

[3] Moisture contents of FFSB 10.16, 10.15 and 10.17% for screens size 3.0 x 3.0, 3.0 x 2.5 and 2.5 x 2.5 mm of hammer mill respectively

[4] Moisture contents of diets 11.26, 11.17 and 11.20% for screens size 3.0 x 3.0, 3.0 x 2.5 and 2.5 x 2.5 mm of hammer mill respectively

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Table 4.4 Conditions of processing on the quality of ground soybean, full fat soybean (FFSB) and diets[1]

Items	Raw soybean	Ground soybean particle sizes, µm			FFSB particle sizes, µm			Diet particle sizes, µm		
		428	351	307	945	879	822	778	705	665
Trypsin inhibitor, mg/g[2]	9.42	8.26	8.21	8.15	4.01	4.02	3.97	2.58	2.62	2.47
Urease index										
pH change	2.14	1.16	1.16	1.14	0.07	0.08	0.07	0.02	0.01	0.01
Color value[3]										
L	28.3 ± 0.1	29.0 ± 0.2	28.3 ± 0.1	29.4 ± 0.1	49.5 ± 0.2	49.7 ± 0.1	49.4 ± 0.1	47.3 ± 0.1	47.8 ± 0.1	46.2 ± 0.2
a	47.8 ± 0.2	47.2 ± 0.1	47.2 ± 0.1	47.2 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	10.3 ± 0.1	10.4 ± 0.1	10.3 ± 0.1
b	1.33 ± 0.01	1.39 ± 0.02	1.40 ± 0.01	1.33 ± 0.01	58.3 ± 0.1	57.9 ± 0.1	58.4 ± 0.1	51.7± 0.1	53.2 ± 0.3	52.2 ± 0.1
Colored Spots	TNTC[4]	TNTC[4]	TNTC[4]	TNTC[4]	4	5	4	2	1	1
Protein solubility,%	84.1	83.9	84.0	83.4	73.1	72.4	73.7	65.3	64.3	64.9

[1] 2 observations per means

[2] mg pure trypsin inhibited/g of substrate

[3] 5 sections of petri dish were determined and reported by *L* (lightness), *a* (redness) and *b* (yellowness) with mean ± SE

[4] TNTC mean too-numerous-to-count results

<u>Experiment 2</u>

The average temperature of pen was 23.2 ± 2.9, 30.9 ± 2.2 and 29.9 ± 1.7 °C and the average relative humidity was 86.0 ± 12.7, 44.1 ± 9.5 and 43.0 ± 9.7% at 08:00 am, 12:00 and 17:00 pm respectively. At the age of 35 days, one pig in the 879 μm FFSB particle size group died due to anaphylactic shock induced cardicvascular failure.

Apparent fecal digestibility of nutrients

The significant differences of apparent fecal digestibility were found in DM and CP (*P*<0.001) at 2nd week of experimental period. The digestibility increased while the particle size was decreased (Table 4.6). Whereas the digestibility of all nutrients showed no significant differences (*P*>0.05) at 4th week of experimental period were found (Table 4.7).

<u>Table 4.6</u> The percentage of nutrient digestibility[1] in weaning pigs at 2nd week of experimental period

Items	FFSB particle sizes, μm			SE	*P*-value
	945	879	822		
Dry matter	87.1[C]	87.9[B]	88.9[A]	0.2	<0.001
Crude fat	76.5	77.1	77.3	0.4	0.406
Crude protein	81.2[C]	83.0[B]	83.9[A]	0.1	<0.001
Crude fiber	47.4	48.8	48.2	0.5	0.183
Calcium	82.2	82.6	82.7	0.5	0.715
Total phosphorus	72.9	73.2	73.8	0.3	0.126
Gross energy	87.1	87.5	87.5	0.2	0.239

[1] 4 observations per mean

[A, B, C] Means within the same row with different superscripts differ highly significant (P<0.001).

<u>Table 4.7</u> The percentage of nutrient digestibility[1] in weaning pigs at 4th week of experimental period

Items	FFSB particle sizes, μm			SE	*P*-value
	945	879	822		
Dry matter	91.2	91.3	91.4	0.1	0.436
Crude fat	79.2	79.7	80.0	0.3	0.235
Crude protein	83.9	84.0	84.1	0.2	0.822
Crude fiber	49.2	50.0	49.8	0.4	0.348
Calcium	88.8	88.8	89.3	0.5	0.758
Total phosphorus	74.4	74.5	74.6	0.4	0.974
Gross energy	91.4	91.7	91.7	0.1	0.090

[1] 4 observations per mean

Growth performances

No significant differences on starting weight among treatment groups of the weaning pigs were found. Weaning pigs received FFSB particle size 822 µm gave the best final weight and weight gain (*P*<0.05).

At 1st week of experimental period (24 – 30 days of age), no significant differences in all parameters among treatment groups were found (*P*>0.05).

At 2nd week of experimental period (31- 37 days of age), weaning pigs received FFSB particle size 822 µm gave the best gain:feed ratio (*P*<0.05). Furthermore, weaning pigs received FFSB particle size 879 and 822 µm gave higher ADG than 945 µm (*P*<0.01).

At 3rd – 4th week of experimental period (38 - 51 days of age), no significant differences in all parameters among treatment groups were found (*P*>0.05).

Finally, the overall period of experimental (24 - 51 days of age), weaning pigs received FFSB particle size 822 µm gave the best ADG (*P*<0.05) (Table 4.8).

<u>Table 4.8</u> Effect of full fat soybean (FFSB) particle size on growth performance of pigs

Items	FFSB particle sizes, µm			SE	*P*-value
	945	879	822		
Number of pigs	12	12	12		
Starting weight (kg/pig)	5.96	6.10	6.03	0.07	0.416
Final weight (kg/pig)	15.7^{b}	16.0^{b}	16.8^{a}	0.2	0.028
Weight gain (kg/pig)	9.73^{b}	10.54^{b}	10.75^{a}	0.19	0.023
1st wk of experimental period (24-30 days of age)					
ADFI (g/pig/day)	177	183	181	12	0.919
ADG (g/pig/day)	141	145	163	10	0.352
Gain:feed	0.79	0.78	0.90	0.03	0.058
2nd wk of experimental period (31-37 days of age)					
ADFI (g/pig/day)	337	336	364	9	0.133
ADG (g/pig/day)	270^{B}	308^{A}	328^{A}	8	0.007
Gain:feed	0.80^{b}	0.86^{ab}	0.90^{a}	0.02	0.025
3rd wk of experimental period (38-44 days of age)					
ADFI (g/pig/day)	568	598	587	22	0.643
ADG (g/pig/day)	415	435	464	14	0.153
Gain:feed	0.74	0.73	0.79	0.04	0.522
4th wk of experimental period (45-51 days of age)					
ADFI (g/pig/day)	758	761	776	16	0.720
ADG (g/pig/day)	547	560	581	17	0.679
Gain:feed	0.74	0.74	0.75	0.03	0.914
Overall period of experimental (24-51 days of age)					
ADFI (g/pig/day)	460	478	480	8	0.378
ADG (g/pig/day)	348^{b}	359^{b}	384^{a}	7	0.023
Gain:feed	0.76	0.78	0.80	0.02	0.143

$^{A, B}$ Means within the same row with different superscripts differ highly significant (P<0.01).

$^{a, b}$ Means within the same row with different superscripts differ significantly (P<0.05).

CHAPTER V

DISCUSSION

<u>Experiment 1</u>

Physical characteristics

Particle size of ground soybean passed through the splited screens size from 3.0 x 3.0 to 3.0 x 2.5 and 2.5 x 2.5 mm of hammer mill were continuously decreased because of particle size of product was determined by screens size of the opening area in the hammer mill (Crenshaw, 2001). When ground soybeans were passed to extrusion process, FFSB, were lager particle sizes than ground soybeans. This increase was described by Woodroofe (1995) that extrusion process affected particle size of soybean as under the conditions of heat and pressure that occur within an extruder barrel the starch gel once formed and will remain in an elastic viscous state until the product exits the die of the extruder. At this point the sudden drop in pressure will cause the moisture within the product to vaporize into steam and increase dramatically in volume. In addition to increased particle size of FFSB, gelatinization of starch affected hydrogen binding among polysaccharide chains in the granule structure, allowing water to associate with free hydroxyl groups. Swelling and opening of the granule structure were occurred (Serrano, 1997). The particle sizes of the complete diets were smaller than FFSB because of the smaller particle size of other ingredients and FFSB contributed only 25% in the diet. Reducing particle sizes decreased S_{gw}, implying that the uniformity of particle sizes increased. Behnke (1985) suggested that S_{gw} value less than 2.0

enhances the mixing efficiency of mash feed diet. S_{gw} of diet in this study was ranging 2.14 to 2.18 over the suggested value for mixing efficiency of mash diet. However, they were in the normal range of S_{gw} in feed sample (2.0 – 2.4) which was reported by Baker and Herrman (2002). Surface area can be used by an animal nutritionist to determine the rate of digestibility or by a process engineer to calculate grinding efficiency. Grinding of grain occurs under exposure of mechanical forces that trench the structure by overcoming of the interior bonding forces and after grinding the state of the grain is changed in the grain size and the grain shape (Behnke, 1985). Healy et al. (1994) reported that particle size was reduced from 900 to 300 µm, surface area of corn and diet were increased more than doubled from 61 to 141 and 103 to 158 cm^2 /g respectively. In the present study, surface area of ground soybeans were increased 31% from 127 to167 cm^2/g when ground soybean particle sizes were reduced from 428 to 307 µm. Significant difference of surface area among particle sizes were found as same pattern as in surface area of FFSB which were increased 11.5% from 60.0 to 66.9 cm^2/g when FFSB particle sizes were reduced from 945 to 822 µm.

In the present study, no significant differences were found in the angle of repose of FFSB when particle size decreased whereas the angle of repose in ground soybean was significant differences. The angle of repose was determined flow ability during processing and affected by moisture content (Wang et al., 1995) and particle size (Lawrence et al., 2003). The moisture contents of ground soybeans and FFSB in this study were nearly the same among groups thus moisture was dismissed. In the case of particle size, Lawrence et al. (2003) reported that reducing particle size of extruded-expelled soybean meal from 965 to 639 µm and solvent-extracted soybean meal from 1,226 to 444 µm increased the angle of repose 17.4 and 20.7% respectively. The angle of repose in FFSB and diet did not show significant differences among particle sizes because of the range between particle sizes were too small. It was only 6.9 and 6.5% for FFSB and 9.4 and 5.7% for diet compared with ground soybean 18.0 and 12.5% when screen sizes of hammer mill were decreased from 3.0 x 3.0 to 3.0 x 2.5 and 3.0 x 2.5 to 2.5 x 2.5 mm respectively. However, this study did not have any problems with flow ability during FFSB processing. Wang et al. (1995) reported that moisture content and particle size of soybean meal had significant effects on bulk density. They also reported that fine particle sizes (soybean meal passed through U.S. no.16 sieve or less than 1,191 μm) gave higher bulk density than coarse particle sizes (soybean meal retained on U.S. no.16 sieve or more than 1,191 μm) and increasing moisture content of soybean meal, bulk density was decreased. Bulk density of FFSB and diet particle size showed no significant differences. Mani et al. (2006) reported that reducing particle sizes of corn stover from 412 to 262 and 193 μm did not significant difference in bulk density. The differences in size of particles in ground soybean, FFSB and diet in this study were not significant enough to affect bulk density.

Production cost

Energy consumption of grinding process was increased 82.0 and 97.5% when reducing particle size from 428 to 351 and 307 μm but no significant differences between ground soybean particle size 351 and 307 μm. The increasing in fine grinding energy consumption resulted from hard and rigid texture of raw soybean until met the required particle size. For production rate of grinding process was decreased 17.5 and 47.4% when FFSB particle size reduced from 428 to 351 and 307 μm. This decrease in fine production rate of grinding process resulted from higher dust levels (Goodband et al., 2002). Similar result was reported by Wondra et al. (1995) that reducing corn particle size from 1,000 to 800, 600 and 400 μm, grinding energy consumption was increased 14.8, 40.7 and 200% respectively and production rate was decreased 0, 3.7 and 51.9% respectively when compared with 1,000 μm of corn particle size respectively. For extrusion process, no significant differences for all conditions among particle sizes were found which supported by Garber et al. (1997). They reported that particle size of grain range 100 – 1,000 μm no affect changing operating conditions of extruding process, but each value dropped significantly as the particle size increased more than 1,000 μm. Total electrical energy consumption and production cost of both grinding and extrusion processes showed that no significant differences among particle sizes in this study. However, in economic aspect, the increasing in production cost 6.6 bath/ton is significant to manufactory.

Nutritional content of soybean

In the present study, the nutritional content (DM-basis) of soybean was little changed after grinding and extrusion processes. This was supported by Woodroofe (1995) who found that extrusion has little effect on crude fat, crude fiber and mineral. However, extrusion has affect protein denaturing that some of amino acid chains have been broken up into individual amino acid or into shorter chains. Serrano (1997) found that the extensive lysine loss occurred when soybean was extruded under severe conditions of temperature (>180°C) or shear force (>100 rpm) at low moisture ($\leq$15%). Extruder temperature in the experiment was highest at 118 °C and moisture content of soybean approximately 10.2% thus crude protein was little changed after passed the extrusion process.

Soybean and diets quality

Extrusion process was efficient in the inactivated anti-nutritional factors of soybean depending on barrel temperature (Serrano, 1997). This supported by Zarkadas and Wiseman (2005) reported that trypsin inhibitor contents of soybean ranged from 24.1, 16.8, 6.4 and 2.9 mg/g with increasing extrusion temperature to 70, 90, 110 and 150°C respectively. In the present study, trypsin inhibitor contents of FFSB were 3.97 – 4.02 mg/g at 115 – 118 °C of extrusion temperature. The pH change was closely paralleled by trypsin inhibitor contents and was easier to determine quality soybean meal and widely used to correlate processing conditions with reducing of anti-nutritional content (Fasina et al., 2003). The pH change falls rapidly from approximately 2.14 units as ground soybean to near zero after extruded. The suitable level of urease index by the pH change which was acceptable to most nutritionists for identified good quality soybean meal were in between 0.05 – 0.20 (Dudley-Cash, 1999). Prachayawarakorn et al. (2004) reported that the influence of temperature plays an important role in accelerating the urease inactivation for soybean containing low to moderate moisture contents (13.5 – 36.0%), in particular the reaction rate constant for inactivating urease enzyme were increased as temperature increased from 110 to 150 °C. For changing in color and red color spots showed in analysis occurred from ammonia content which was released from the chemical reaction between the urea and residual urease enzyme in unsuitable processed soybean and urea in the solution which were measured by changing in color of indicator (Juttupornpong, 1996). Ground soybean which received insufficient heat treatment to destroy enzyme urease in raw soybean gave the highest of *a* color value (redness) and red color spots. After ground soybean was passed continuously to extrusion process at temperature 115 – 118 °C number of red color spots were rapidly decreased. In addition to the protein solubility was also an important parameter that characterizes the protein quality of soybean. Dudley-Cash (1999) and Araba and Dale (1990) suggested that protein solubility between 70 – 75% and 70 – 85% were indicative of adequately processed soybean. In the present study, protein solubility of raw soybean was 84.1%, decreased only little after grinding (83.4 – 84.0%) and decreased after extrusion process (72.4 – 73.7%). The reduction in protein solubility described by Prachayawarakorn et al. (2004) that the disulfide bond of proteins which links between amino acid groups was destroyed therefore the proton of hydrogen in water molecules inside proteins reacted with the sulfur of disulfide to form as sulfhydryl and protein molecules aggregated. The magnitude of its reduction depended on the heating process and operating conditions such as temperature and time thus it is the truth that it can occur to the other amino acids (Calvalho and Sgabieri, 1997; Dudley-Cash, 1999).

<u>Experiment 2</u>

Nutrients digestibility and growth performances

The digestive tract of early-weaned piglet is immature at this age and is not entirely adapted to digest vegetable raw materials. One of the most important factors in causing digestive disturbance is the low activity of proteolytic enzymes (Cramwell, 1995). Reducing particle size increased the surface area of the ingredient available to the digestive enzymes (Goodband et al., 2002). In the present study, it was found that weaning pigs at 2nd week of experimental period received FFSB particle size 822 μm had the best apparent fecal digestibility of DM and CP when compared to the other (*P*<0.001). The improvement of digestibility due to reducing particle size agrees with

Fastinger and Mahan (2003) showed that apparent digestibility of alanine, serine and proline increased also true digestibility of valine, methionine and alanine increased as two source of soybean meal particle size (900 and 800 μm) were compared. However, there was limited information of soybean particle size on animal but effects of cereal particle sizes reported in several experiments. Owsley et al. (1981) reported that reduction in particle size of sorghum from 1,200 to 802 to 471 μm improved the apparent digestibility of DM, starch, N and GE measured at the terminal ileum and for the total digestive tract of growing pigs. Giesemann et al. (1990) observed improvements digestibility of DM, N and GE of corn based diet fed to growing-finishing pigs as particle size was reduced from 1,506 to 641 μm. Researchers generally agree that nutrient digestibility increased as particle size decreased but the nutritional implications of particle size uniformity were not fully understood. Wondra et al. (1995) reported that increased digestibility of DM, N and GE due to increased in particle size uniformity or decreased in standard deviation from 2.5 to 1.7 and from 2.6 to 1.8 as particle size decreased from 1,020 to 450 and from 1,017 to 517 μm for corn and diet after mixing respectively. They suggested that increased nutrient digestibility by reduced particle size may result both from increased surface area and increased uniformity of particle size. The S_{gw} in the present study was an acceptable range and showed the positive digestibility of DM and CP.

Increasing nutrient digestibility can lead to an increase in growth performance of piglets. Improvement of growth performance has been observed with reducing particle size of grains for nursery pigs (Wu and Fuller, 1974; Healy et al., 1994). Wu and Fuller (1974) found a positive response in ADG and FCR for the first week after weaning when the screen size used for maize grinding was decreased from 9.5 to 1.6 mm. However, no significant difference was found when maize grinding was decreased from 4.0 to 1.6 mm. Healy et al. (1994) reported that reducing the particle size of maize from 900 to 300 μm improved ADG and FCR for the first two weeks after weaning but not afterward. In the present study, it was found that weaning pigs received FFSB particle size 822 μm gave the best ADG (P<0.01) and gain:feed ratio (P<0.05) at 2nd week of experimental period. For the overall period of experimental significant showed only in ADG. It could be due to increasing in apparent DM and CP digestibility and the low trypsin inhibitor contents in FFSB which supported by Chang et al. (1984). They suggested that FFSB do not exceed a maximum level of 5.0 mg/g, have been shown to have little or no effect on nutrient digestibility. This suggestion was confirmed by Zarkadas and Wiseman (2005) that the daily live weight gain and feed conversion ratio improved continuously from 413 to 539 g and 1.82 to 1.48 respectively when weaning pigs received trypsin inhibitor content in extruded FFSB decreased from 24.1 to 2.9 mg/g or extrusion temperature increased from 70 to 150 °C. In the present study, trypsin inhibitor contents of FFSB were 3.97 – 4.02 mg/g at 115 – 118 °C extrusion temperature. From the mention research, it could conclude that no effect of trypsin inhibitor contents on nutrient digestibility and growth performance in this study. Therefore, the improvement of ADG and gain:feed ratio should be due to the reducing in particle size, increased surface area and uniformity available to the digestive enzyme and increased apparent fecal digestibility of nutrients.

In conclusion, the studies demonstrated that reducing in particle size of FFSB to 822 μm increased uniformity of particle size or decreased S_{gw} while surface area was increased. Reducing particle size increased energy consumption, decreased production rate of grinding process but did not affect total energy consumption and production cost to produce FFSB. There was little changed in the urease index (pH change and color value), protein solubility and trypsin inhibitor value of ground soybean, FFSB and diet among particle sizes. Reducing particle size increased apparent DM and CP digestibility at 2^{nd} week of experimental period and showed positive impact on gain:feed ratio at 2^{nd} week and ADG at 2^{nd} week and the overall period of the experiment.

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