A Dissertation

for the Degree of Doctor of Philosophy

Effects of Dietary Tryptophan Levels on Performance, Blood Metabolites and Nutrient Digestibility in Sows

사료 내 다른 수준의 트립토판이 모돈의 번식 성적, 혈중 대사물, 영양소 소화율에 미치는 영향

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Overall Summary

Effects of Dietary Tryptophan Levels on Performance, Blood Metabolites and Nutrient Digestibility in Sows

The objectives of this research described in this dissertation were 1) to evaluate effects of dietary Trp levels during gestation and lactation on performance of sows, 2) to determine the optimal relative Trp level to Lys gestating and lactating sows, and 3) to estimate the nutrient and AAs digestibility in sows by varying Trp/Lys ratios.

Experiment Ⅰ. Effects of Dietary Tryptophan Levels in Gestating Sows on Performance and Blood Metabolites

This study was conducted to evaluate the dietary Trp levels in gestating sows on performance and blood metabolites. A total of 45 F1 multiparity sows (Yorkshire×Landrace) with average body weight (BW) of 218.96±2.43 kg with a parity of 4.8±0.03 was allotted to one of three treatments by parity, BW and backfat (BF) thickness in completely randomized design after artificial insemination service. Each treatment was designed based on relative ratio Trp to Lys in the gestation diet. Treatments were as followed: 1) Trp18%: 18% *ai* (apparent ileal digestible) Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. As fed basis, experimental diet contained 2,333 kcal NE/kg, 12.00% crude protein (CP), 0.501% *ai* Lys, 68% *ai* TSAA/*ai* Lys, and 72% *ai* Thr/*ai* Lys as fed basis. All other nutrients in experimental diet were met or exceeded the NRC requirement (2012). Feed intake of experimental diet at the 2nd and the 3rd parity of gestating sows was 2.2 and 2.4 kg/d, respectively. During gestation and lactation, there were no significant differences in BW, BF thickness and their changes among treatments. In farrowing performance, Trp18% treatment had higher number of total born and born alive than other treatments (P=0.07 and P=0.06, respectively). High level of Trp decreased the number of piglets in total born and born alive (linear, P=0.06). However, the number of

stillborn and mummy showed no differences among treatments. Although birth weight of piglets among treatments similar, total litter weight was decreased linearly as dietary Trp level was increased (linear, P=0.07). After cross-fostering, litter weight, litter weight gain, piglet weight and piglet weight gain were not affected by dietary treatments. In addition, dietary Trp levels during gestation did not influence on average daily feed intake (ADFI) during lactation and weaning to estrus interval (WEI) of sows. However, ADFI of Trp22% and Trp26% treatments were higher than Trp18% treatment at d 10 and 11 of lactation (P<0.10). In blood metabolites, Trp22% treatment had the highest blood urea nitrogen (BUN) concentration on d 35 of gestation (P=0.03; quadratic, P=0.01). Trp22% treatment tended to decrease blood cortisol level (P=0.07) and cortisol level showed a quadratic response to dietary Trp level on d 35 of gestation (P=0.06). High dietary Trp treatment had higher melatonin level on d 35 of gestation (P=0.02) and it showed the linear effect as dietary Trp level (P<0.01). Serotonin level showed no difference among treatments during whole experimental period. At d 35, 70, and 110 of gestation, high Trp level in gestation diet showed higher Trp concentration (linear, P<0.01; P=0.01; P<0.01, respectively) and relative Trp level to large neutral amino acids (LNAA, linear, P<0.01; P<0.01; P=0.03, respectively) of blood plasma. However, at 24 h postpartum and d 21 of lactation, there were no linear effect on Trp concentration (P=0.19; P=0.37, respectively) and relative Trp level to LNAA (P=0.60; P=0.58, respectively) although dietary Trp level for gestating sows was increased. No differences were observed in casein, fat, protein, lactose, total solid and solid not fat content of sow milk on colostrum and milk (d 21 of lactation) among treatments. Furthermore, milk production, milk dry matter (DM), and milk energy of sow milk were not affected by Trp level in gestation diet. There were no significant differences in protein, fat, and energy contents of sows and their changes during gestation and lactation. Consequently, this study demonstrated that high Trp concentration and Trp/LNAA level in blood during gestation did not show beneficial effects on farrowing performance when sows were fed diets containing *ai* Trp/ai Lys above 22%. Moreover, the piglet performances of sows were not improved in despite of high feed intake at d 10 and 11 of lactation. It represented that maximal level of *ai* Trp/*ai* Lys in gestation diet was 18% (based on 0.50% *ai* Lys in diet and 2.4 kg daily feed intake and maximal dosage of daily *ai* Trp intake may be 2.16 g during gestation.

Experiment Ⅱ. Effects of Dietary Tryptophan Levels in Lactating Sows on Performance and Blood Metabolites

This experiment was conducted to evaluate the effects of dietary Trp levels in lactating sows on the reproductivity and blood metabolites. A total of 30 mixed-parity (average 4.73) lactating sows (Yorkshire×Landrace) with an initial BW of 234.27±17.35kg were used in a 3 week trial. Sows were allotted to one of three treatments in a completely randomized design by their BW, BF thickness, parity and litter weight. Each treatment was designed based on relative ratio Trp to Lys in the lactation diet. Treatments were as followed: 1) Trp18%: 18% *ai* Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. Experimental diets contained 2,350 kcal NE/kg, 13.5% CP, 0.65% *ai* Lys, 62% *ai* TSAA/*ai* Lys, and 75% *ai* Thr/*ai* Lys, as fed basis. All other nutrients in experimental diet were met or exceeded the NRC requirement (2012). Although Trp22% and Trp26% treatments showed higher daily feed intake in the middle of lactation (8 ~ 14 d) than Trp18% treatment, there were no differences in BW, BF thickness, ADFI, and WEI among treatments during the whole lactation. At farrowing, dietary Trp levels did not affect number of piglets in total born, still born, mummy and born alive. After cross-fostering, Trp22% and Trp26% treatments showed the higher litter weight at d 21 of lactation compared with Trp18% treatment (P=0.08). In addition, the linear increases were observed in litter weight at d 21 of lactation and litter weight gain as dietary Trp level was increased ($P=0.06$ and $P=0.04$, respectively). But, piglet BW and BW gain during lactation were not affected by dietary Trp level. In blood metabolites of sows, there were no differences in the concentration of BUN, cortisol, and melatonin among treatments. Higher serotonin concentration was observed in Trp22% and Trp26% treatments than Trp18% treatment at 24 h postpartum (P=0.03). As dietary Trp level increased, serotonin concentration at 24 h postpartum increased (linear, P=0.02). At 24 h postpartum, blood cortisol tended to decrease whereas blood melatonin was numerically increased as dietary Trp level of lactation diet was increased (P=0.56 and P=0.16, respectively). In addition, BUN, cortisol, and glucose level in blood of nursing piglets were not affected by dietary Trp level in lactation sow's diet. Trp concentration and Trp/LNAA level of blood had no difference among treatments. No differences were

observed in casein, fat, protein, lactose, total solid and solid not fat content of sow milk among treatments. Furthermore, milk production, milk DM, and milk energy were not affected by dietary Trp level in lactation. There were no significant differences in protein, fat, and energy contents of sows and their changes during lactation. As a consequence, high dietary Trp level in lactation feed (above 22% *ai* Trp/*ai* Lys, 0.65% *ai* Lys) increased the serotonin level of blood at farrowing and improved litter weight gain with elevating feed intake in the middle of lactation. Nevertheless any improvements were not found in BW and BF thickness change of sows, piglet BW gain, ADFI, and WEI when sows were fed diets containing *ai* Trp/*ai* Lys above 22% during the whole lactation. These results indicated that maximal ratio of *ai* Trp/*ai* Lys in lactation diet was 18% (0.65% *ai* Lys in lactation feed).

Experiment Ⅲ. Effects of Dietary Tryptophan Levels in Gestating Sows on Nutrient and Amino Acids Digestibility

This experiment was conducted to evaluate the effect of dietary Trp levels on nutrient digestibility in gestating sows. A total of 9 multiparous gestating sows (Yorkshire × Landrace) with an initial BW of 255.84 ± 20.84kg was used in a digestibility trial. Sows were allotted to one of three treatments in a completely randomized design by their BW, BF thickness, and their parity. Each treatment was designed based on relative ratio Trp to Lys in the gestation diet. Treatments were as followed: 1) Trp18%: 18% *ai* Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. An experimental diet contained 2,333 kcal NE/kg, 12.00% CP, 0.501% *ai* Lys, 68% *ai* TSAA/*ai* Lys, and 72% *ai* Thr/*ai* Lys, as fed basis. All other nutrients in experimental diet were met or exceeded the NRC requirement (2012). Each treatment diet was provided 2,400 g/d once daily to gestating sows. There were no differences in BW, BF thickness, and their changes during digestibility trial. In nutrient digestibility, dietary Trp levels showed no effect on digestibility of DM, CP, fat and ash (P=0.73, P=0.98, P=0.41, and P=0.13, respectively). In addition, there was no significant difference in nitrogen retention. Except Trp and Ile, dietary *ai* Trp/*ai* Lys level over 18% had no effect on the other amino acids digestibility. Trp digestibility was improved linearly (P=0.04) as *ai* Trp/*ai* Lys level was increased and Trp

digestibility of Trp26% treatment was higher than those in Trp18% and Trp22% treatments (P=0.09) whereas, digestibility of Ile was decreased (linear, P=0.05). Although Ile digestibility was reduced by increasing dietary Trp level, this experiment represented that nutrient digestibility and nitrogen retention were not different when sows were fed diets containing *ai* Trp/*ai* Lys above 18%.

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List of Abbreviations

AAs	amino acids
ADFI	average daily feed intake
ai	apparent ileal digestible
BCS	body condition score
BF	backfat
BUN	blood urea nitrogen
BW	body weight
CP	crude protein
DM	dry matter
FFA	free fatty acid
FU	feed unit
IDO	indoleamine 2, 3-dioxigenase
LNAA	large neutral amino acid
Lys	lysine
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NE	net energy
SID	standardized ileal digestible
TDO	tryptophan dioxygenase
Thr	threonine
Trp	tryptophan
TSAA	total sulfur amino acid
WEI	weaning to estrus interval

Chapter Ⅰ. General introduction

Tryptophan (Trp) is one of the aromatic amino acids (AAs) and was discovered at 1901 almost 100 years ago when it was isolated from a pancreatic digest of casein (Figure 1). Trp is known to have many metabolic roles besides being an essential component of body muscle tissue (Sidransky, 1985). Unlike other aromatic AAs (phenylalanine, tyrosine), Trp contains an indole ring (Amy, 2002).

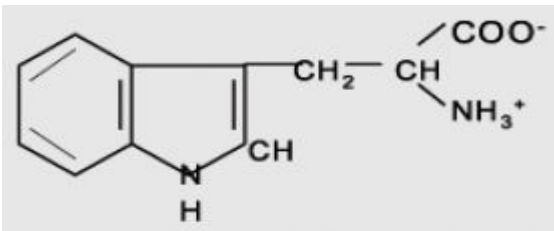


Figure 1. Structure of tryptophan

With the Lys, Trp is mostly to be second deficient in diet mainly based on common maize (Table 1) (ARC, 1981; Lewis, 1985). The effects of deficiency are slow growth and eye cataracts. Trp can be converted by the tissues of the pig to the niacin (vitamin B3), so it is important to provide adequate niacin to minimize the Trp requirement (Fond and Maner, 1984). In Korea, diets for pigs are corn-based in which Trp is often considered the second limiting amino acid. Limiting Trp levels in pigs' diets have been shown to have a negative effect on growth performance (Henry, 1995). Uttecht et al. (1991) suggested that Marginal or limiting Trp levels in swine diets have resulted in negative effect on feed intake in young pigs definitely.

Trp has various roles such as serves as a subunit of protein, and precursor of numerous metabolites including the neurotransmitter serotonin (5-hydroxytryptamine), tryptamine, the hormone melatonin and niacin. In the brain, Trp is the precursor for serotonin, which is thought to inhibit a variety of behaviors such as aggression, feed intake and stress response by reducing stress hormones (Laycock and Ball, 1990; Chung et al., 1991; Shea et al., 1991; Adeola and Ball, 1992; Adeola et al., 1993; Markus et al., 2000; Lepageet al., 2003;

Koopmans et al., 2005; Zhang et al., 2007; Poletto et al., 2010). The uptake of Trp into the brain is dependent on the availability of the transport system referred to as the blood-brain-barrier (Pardrodge, 1998; Markus et al., 2000, Amy, 2001). The transport system only allows passage from blood to the brain and also is used by large neutral amino acids (LNAA; Ile, Leu, Val, Phe, and Tyr). The LNAA compete with Trp for transportation and this competition can affect the amount of Trp in the brain.

In addition, Trp is a precursor for the synthesis of acetyl-CoA. L-dietary Trp is easily absorbed from gastrointestinal tract and metabolized to serotonin, other metabolites and excreted in the urine (Heine et al., 1995, Yao et al., 2011). The various animals and feeding phase have their own specific ability to utilize D-Trp. Pigs can less utilize D-Trp than the L-form. Estimating with growing pigs of the biological activity of D-form relative to L-form is from 60% to 100% (Baker et al., 1971; Arenstone and Zimmerman, 1985; Schutte et al., 1988). However, most of feed-grade Trp (95.8%) is L-form in present.

Table 1. Limiting amino acids in cereal grains for swine

Cereal grains	**First**	**Second**	**Third**
Maize	Lysine and tryptophan		Threonine
Barley	Lysine	Threonine	Histidine
Wheat	Lysine	Threonine	-
Sorghum	Lysine	Threonine	Tryptophan
Triticale	Lysine	Threonine	-

(Adapted from Lewis, 1985)

Many researchers thought that multi-functionality of Trp may be helpful to the sow which is always the stress occurring point the gestation and lactation (Li et al., 2011; Moehn et al., 2012, Rosangela Poletto et al., 2014). During the gestation, sow is suffer from the hunger derived from restricted feeding system, movement limitation by stall housing and mixing stress of group housing at early phase after insemination under the group housing system. It was demonstrated that sows are under severe catabolic status during late phase of gestation causing increased oxidative stress (Flowers B et al., 1990). Regardless of sow parity, protein deposition was greater in late than early gestation and fetal growth associate with more to amino acid requirements in late gestation (Soenke M. et al., 2012). Fetal growth

occurs predominantly in late gestation (McPherson et al., 2004).

Srichana (2006) found no significant difference in the Lys requirement of 15.0 g/d between early and middle gestation, but reported an increased requirement of 18.0 g/d in late gestation. Samuel et al. (2010) showed that the Lys requirement of 2nd parity sows was 13.1 g/d and 18.7 g/d in early and late gestation, respectively. In 3rd parity sows, total Lys as the dietary was 8.2 g/d and 13.0 g/d for early and late gestation, respectively. Levesque et al. (2011) found that second parity sows required 7.2 g/d total threonine in early gestation (d 35 – 53) and 13.6 g/d threonine in late gestation (d 92 – 110), based on indicator amino acid oxidation. In multi-parities sows, the total threonine requirement was more than doubled from 5.0 g/d in early gestation to 12.3 g/d in the last third of gestation. Moehn, S. et al. (2012) suggested that the Trp requirement of 2nd parity sows increased from 1.7 g/d to 2.6 g/d from early to late gestation.

Due to the negative nutrient balance after farrowing, lactating sow is the catabolic condition (S.W. Kim et al. 2013). Catabolic status in sow increases the production of reactive oxygen species (ROS) causing increased oxidative stress (Bernardi F et al, 2008, Berchieri-Ronchi CB et al., 2011). Oxidative stress is related to the health status in swine. High oxidative stress in sows caused the impaired milk production, reproduction performance and longevity of sows (Flowers B et al., 1990; Zhao Y, 2011a; Zhao Y, 2011b).

For the reducing catabolism of lactating sow originated from high milk production, a rapid increase in daily feed intake is important at lactating sow (Noblet et al., 1990). Insufficient feed intake during lactating is caused to greater BW loss, decreased milk production and reproductive problems that may lead to culling of the sow (Baidoo et al., 1992; Eissen et al., 2000).

The objectives of this research described in this dissertation were 1) to evaluate effects of dietary Trp levels during gestation and lactation on performance of sows, 2) to determine the optimal relative Trp level to Lys gestating and lactating sows, and 3) to estimate the nutrient and AAs digestibility in sows by varying Trp/Lys ratios.

Chapter Ⅱ. Review of Literatures

Trp, same as the other essential amino acid (Lys, threonine), is an important substrate for protein synthesis. However, that is not the only function. It serves as the precursor of serotonin, melatonin, tryptamine, NAD (nicotinamide adenine dinucleotide), and NADP (nicotinamide adenine dinucleotide phosphate), as well as meeting the majority of the requirement for nicotinic acid (Sainio et al., 1996). By its involvement in such diverse pathways, Trp and its metabolites regulate neurobehavioral effects such as appetite, sleeping-waking-rhythm, impulsivity, aggression, sexual behavior, and pain perception (Leathwood, 1987; Baranyiova, 1991; Amy, 2002).

Serotonin is synthesizes in the brain and gastro-intestinal tract, which plays a very important role in feed intake. Trp also stimulates the release of the hormone Ghrelin from cells on the stomach and pancreas, Ghrelin stimulates the feeling of hunger (Inui A. et al., 2004; Sakata I and Sakai, 2010; Dickson SL et al., 2011).

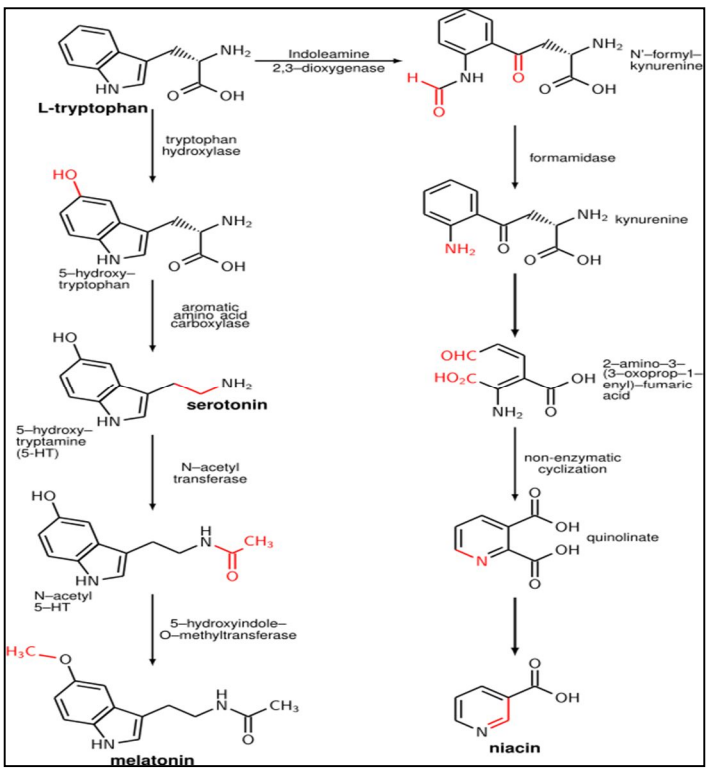
1. Metabolism of tryptophan

Trp is associated with many pathways that yield a variety of end products (Figure 2). A small proportion of Trp serves as the precursor for the neurotransmitter and vasoconstrictor, serotonin. Serotonin is responsible for smooth muscle contraction as well as affecting various other physiological functions. Production of serotonin occurs in the brain stem (1 - 2% of the total body serotonin) and in serotonergic nerves, enterochromaffinic cells, thrombocytes, and mast cells. It is also widely distributed in the hypothalamus (Guyton and Hall, 1996).

1-1. Serotonin

Serotonin is produced by the hydroxylation of Trp (by Trp hydroxylase) in position 5 of the indol-ring, resulting in the formation of 5-hydroxytryptophan, which is then converted by decarboxylation to 5-hydroxytryptamine (or serotonin). Subsequent

metabolism of serotonin results in the formation of melatonin, a neurohormone in the pineal body.



(adapted from Wikipedia)

Figure 2. Metabolic pathway of L-tryptophan to serotonin, melatonin, and niacin

Quantitatively, the other pathway for Trp metabolism after protein synthesis, it is the kynurenine pathway (via tryptophan 2, 3-dioxygenase), which is responsible for over 90% of Trp catabolism. Metabolites from this pathway include: nicotinic acid ribonucleotide (where ≈60 mg of Trp are equivalent to 1 mg of nicotinic acid), picolinic acid, tryptamine, anthranilic acid, kynurenic acid, and acetyl-CoA.

Trp originating from food proteins and from endogenous sources is degraded to indole, skatol, indolacetate, indolpyruvate, and indican when subjected to microbial degradation in the large bowel. At last, serotonin can be metabolized to 5-hydroxyindole acetic acid by the enzyme monoamine oxidase, which is excreted in the urine. However, this

pathway excretes only about 1% of the ingested Trp (Heine et al., 1995; Sainio et al., 1996).

Brain neurotransmitters are intricately linked with Trp and its role in body metabolism. Because of its involvement with brain serotonin, Trp has been shown to be responsible for affecting mood regulation, feed intake, behavior, and sleep patterns (Leathwood, 1987; Baranyiova, 1991). The transport of Trp through cell membranes is competitively inhibited by LNAA such that the Trp/LNAA ratio in plasma is critical point in Trp crossing the blood-brain-barrier and thus being available for serotonin synthesis.

Because serotonin does not pass the blood-brain-barrier, the only way to increase the concentration of serotonin is treatment with its precursors, 5-hydroxytryptophan or Trp, which acts to increase the cerebral serotonin state. Ingestion of meals rich in carbohydrates may increase plasma Trp and consequently, Trp availability to the brain, while meals rich in protein may lower Trp availability to the brain. This situation is occurred due to the carbohydrate-induced release of insulin, which results in an increased uptake of free plasma AAs into muscle tissue, with the exception of the serum albumin-bound proportion of Trp.

Trp is the only amino acid that binds to serum albumin to a high degree, where 90% is bound and 10% is free. The protein-bound proportion of Trp may then be released during the perfusion of the brain capillaries, leading to an increased Trp/LNAA ratio resulting in enhanced transport of Trp into the brain tissue (Heine et al., 1995). Histidine, methionine, and threonine also have been shown to have a negative effect on Trp uptake into the brain (Sainio et al., 1996; Sève, 1999).

Henry et al. (1992) reported that brain serotonin and plasma Trp/LNAA were positively correlated. This relationship agrees with previous studies in rats that also suggest a positive correlation between Trp/LNAA ratio and its effect on brain serotonin content (Fernstrom and Wurtman, 1972; Leathwood, 1987; Yokogoshi et al., 1987, Henry et al., 1996, Sève et al., 1999; Pastuszewsaka et al., 2007).

1-2. Melatonin

Trp is not only metabolized to serotonin, but subsequent metabolism of serotonin results in the formation of melatonin. Melatonin, a neurohormone, is produced in varied amounts depending on the body's cycle. It is involved in the regulation of the day- and night-

biological rhythm, which is affected by light. Melatonin production is increased at night, sometimes ten-fold, relative to daylight hours.

It modulates a range of physiological functions, with the known and studied being sleeping (Lydic and Baghdoyan, 1999) and less studied being an intracellular scavenger of hydroxyl- and peroxide-radicals (Heine et al., 1995) and hair follicle development (Rouvinen et al., 1999). Because of the relationship of Trp to serotonin and melatonin, there have been numerous studies conducted in humans involving oral Trp supplementation as a sleep aid (Anderson and Hrboticky, 1986).

The gastrointestinal tract contains at least 400 times more melatonin than the pineal gland (Huether, 1993). Plasma levels of melatonin as well as GIT tissue levels can rise in response to feeding in pigs (Bubenik et al, 1996). In addition, melatonin was found to affect peristalsis. According to Bubenik et al, (1996), it can be speculated that the increase in melatonin levels after the intake of food may serve as a signal for the regulation of appetite, or for the synchronization of the feeding and digestion processes.

Additionally, Sève et al, (1999) reported that gastric emptying is significantly enhanced in pigs fed with a Trp adequate diet (0.26% total of the diet) compared with a Trp deficient diet (0.16% total of the diet). These results suggest that the Trp effect on feed intake, whether it is through serotonin, melatonin or others, may not only be located in the brain.

2. Tryptophan on feed intake in pigs

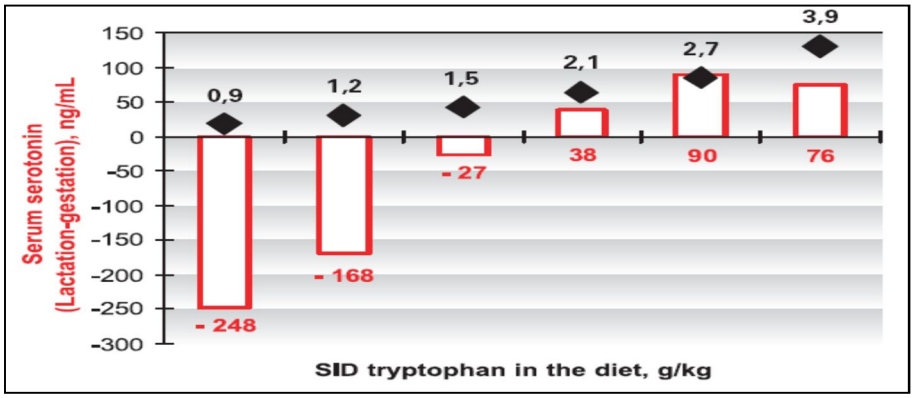
Feed intake is an essential driving force for growth of pig. In a number of studies including piglets (Sève et al, 1991), growing pigs (Henry et al, 1996, Eder et al, 2003), finishing pigs (Henry and Sève, 1992) and lactating sows (Paulicks et al, 2006), an inadequate supply of Trp resulted in a markedly reduced feed intake. Dapoza (2009) suggested that application for the Trp in the commercial unit could be in the piglet and lactating sows where feed intake is the limited phase.

G. W. Libal et al., (2007) studied the Trp level (0.12%, vs. 0.17%, Trp/Lys ratio; 16.0% vs. 22.6%) of lactating sows fed diets supplemented with crystalline Lys to contain

0.75% Lys. Trp level in the lactation diet did not affect number or weight of pigs at d 21. Sows fed 0.17% Trp were heavier at d 21 of lactation than those fed 0.12% Trp due to less weight loss during the 21-d lactation. Feed intake was greater for sows fed 0.17% Trp than for sows fed 0.12% Trp at the 3rd week and overall period. Hence, lactating sows allowed *ad libitum* access to diets containing 22.6% Trp/Lys ratio benefited from higher feed intake and lower weight loss than 16.0% Trp/Lys ratio.

Stahly et al. (1990) and Tokach et al. (1991b) have reported increases in sow reproductivity due to feeding higher crude protein (CP) levels during lactation. Increased feed intake due to increasing dietary protein has been reported by Mahan and Grifo (1975), Mahan and Mangan (1975), and NCR-42 Committee (1978) for lactating sows. In contrast, Johnston (1991) reported increasing levels of protein from 13.6 to 19.2% did not affect feed intake of high-producing sows, but a correlation between increasing dietary protein and reduction of sow weight loss was observed.

In the view of BW loss of lactating sows between Trp level in the fed, Roth-Maier et al, 2004 studied about Trp level (SID (standardized ileal digestible) Trp, 0.09%, 0.12%, 0.15%, 0.21%, 0.27%, 0.39%; SID Lys level, 0.88%) in lactating sows. They showed that increasing SID Trp concentrations in the diet from 0.9 to 2.1 g/kg feed divides weight loss by 3 and increases 2 times feed intake. In parallel, the difference in blood serum serotonin between 28 d of lactation and 85 d of gestation is significantly impacted by Trp content of the diet. Increasing ileal standardized digestible Trp levels from 0.9 g/kg to 2.1 g/kg prevents a decrease in blood serum serotonin between gestation and lactation (Figure 3) which may explain at least part of the effect of dietary Trp on feed intake. Also, Muns et al, (2010) reported similar results. The multi-parities sows fed the Trp enriched diets (Trp=0.26%) showed numerically lower BCS (body condition score) and BF loss (8 and 15% respectively) than control treatment (Trp; 0.20%). On the contrary, there were no significant differences between treatments were observed for BCS, BF thickness and feed intake in sows (Muns R. et al, 2010). At the growing pig, Li et al., (2006) reported that feed intake of 2 or 4 times higher Trp supplemented treatments were not differ with control treatment. However, decreasing of feed intake due to marginal or limiting Trp levels have been reported in growing swine by Lin et al. (1987) and Uttecht et al. (1991).



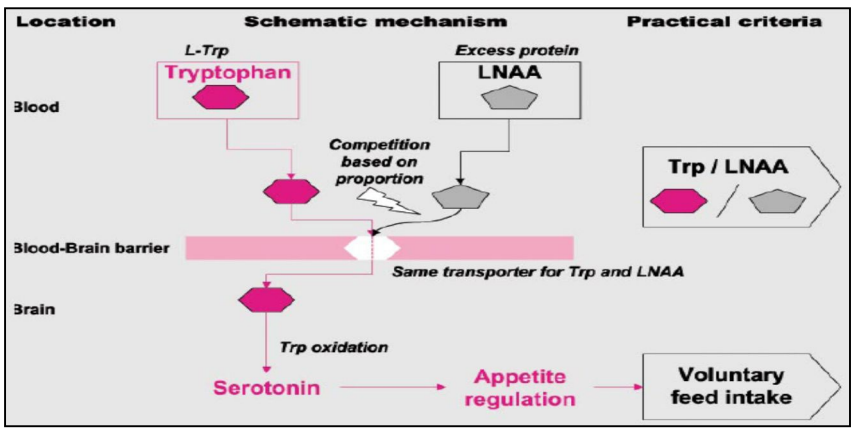
(adapted from Roth-Maier et al, 2004)

Figure 3. Dietary SID tryptophan on the difference of serum serotonin between 85 d of gestation and 28 d of lactation

Zhang et al. (2007) concluded that increased Trp ingestion increased the ghrelin expression in the stomach and ultimately the ghrelin level in the plasma. Ghrelin (peptide of 28 AAs) is an appetite stimulating hormone produced and secreted by the stomach and the duodenum (Inui et al., 2004; Salfen et al., 2004; Zhang et al., 2007). It is also a growth hormone secretagogue, which is involved in numerous functions such as protein synthesis, bone growth and muscle growth. This causes an increased feed intake by the pig to satisfy the hunger feeling. An increased feed intake causes to increased growth and improved feed conversion. Increased feed intake resulted in decreased sow weight loss in the previous study. Thus, Harrell and Arthur (1989) reported that increasing feed intake caused a reduction of sow weight and BF loss and increased milk production. Concomitant increases in both amino acid and energy intake contributed to the decreased sow weight loss observed in both experiments. Similar results were described by Johnston (1990), who reported a correlation between reduced sow weight loss and increasing protein and energy intake. G. W. Libal et al., (2007) suggested the supplementation of Trp in the diet (0.17% Trp in the diet) showed the lower plasma urea nitrogen than 0.12%. Optimal Trp level in the diet is caused less amino acid catabolism and more correct balance of AAs.

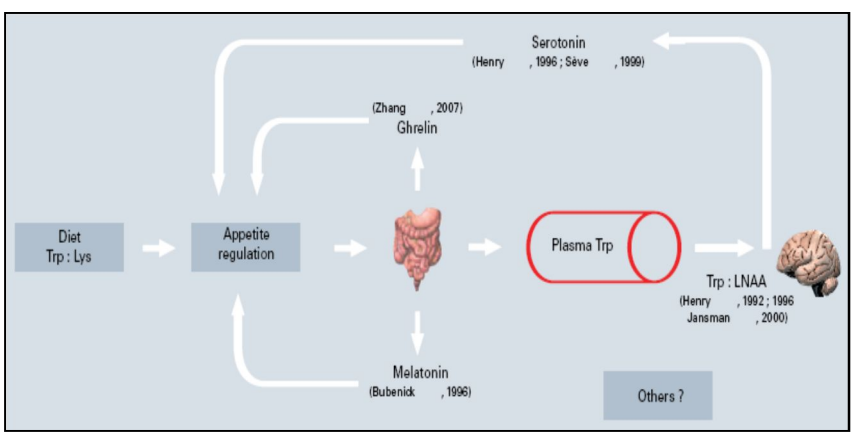
Previous reviewed, LNAA and Trp shared the same transport route through the blood-brain-barrier (competition between Trp and LNAA), the production of serotonin in the brain depends not only on dietary Trp levels, but also on dietary LNAA supply. Therefore,

an excess in dietary LNAA supply induced by a high dietary CPlevel, may impact this central regulation of appetite (Henry et al., 1992; Jansman et al., 2000., Figure 4).



(adapted from Henry and Sève 1993)

Figure 4. Trp and LNAA resulting from excess protein on brain serotonin and appetite



(Adapted from Ajinomoto, 2008)

Figure 5. Possible implication of tryptophan in appetite regulation

Consequently, applying the high level Trp in the diet has several beneficial effects for the increasing feed intake (Figure 5) and preventing of catabolism in the gestating and lactating sows.

3. Tryptophan on stress indicator and behaviors in pigs

Trp has the potential to affect behaviors such as aggression, feed intake, and stress through its impact on serotonin synthesis as well as through its impact on cortisol release (Rodwell, 1979). Cortisol is also used as an indicator of the hypothalamic-pituitary-adrenocortical axis activity in stress eliciting situations (Mormède et al., 2007). Though cortisol secretion is as the defensive strategy of body against stress, the continuous secretion of cortisol during chronic stress is harmful as it leads to immune suppression.

Hence, blocking of cortisol secretion appears to be an ideal approach against to stress. Trp plays a vital role in synthesis of neurotransmitter serotonin. Serotonin in turns directly blocks cortisol in rainbow trout (Lepage et al., 2002).

Trp may have a non-behavioral impact on hair follicle regulation due to it being a precursor to melatonin, which has the potential to block the hormone adrenocorticotropin (ACTH; Ryder, 1973; Rose and Sterner, 1992; Rose, 1995).

In general, pigs are very adaptable to their environment, but behavioral type and social status may impact how they respond to nutritional stimuli. However, there has been limited research conducted in the area of behavior and Trp in swine, and often, much of it is inconsistent with expected metabolite changes (Amy, 2002).

Poletto et al. (2010) studied the effect of additional Trp on the behavior and aggressive in grower gilt. They found that higher level of supplement of Trp in the diet reduced the behavioral activity and the aggressive action of grower gilts. This result may be originated from the increased production of serotonin. Similarly, Y. Z. Li et al., (2006) reported that increasing Trp spent more time lying and less time eating in grow-fisher pig. In addition, dietary Trp decreased the total duration of fighting approximately 50%.

Muns, R et al, (2010) studied the effect of dietary Trp supplementation during lactation on multi-parities sow's behaviors and performance. They reported that higher level of Trp in diet showed lower number of position change at 6 ~ 7 d after farrowing. However, difference between each treatment was not observed for time spent in lying, sitting, and standing.

About the mixing (regrouping) stress, there were different studies. Y. B. Shen, M. T.

Coffey and S.W. Kim (2015) conducted the effects of short term dietary supplementation of L-Trp and reducing LNAA on growth and stress response in nursery and growing pigs. They suggested that short-term supplementation of L-Trp (0.8%) improved growth performance of pigs during period of social-mixing and were associated with reduced stress hormone concentrations in grower pig.

However, Y. Z. Li et al. (2011) reported that supplementation of dietary Trp at 2.3 times (high-Trp treatment; 0.35% in the gestation diet and 0.48% in the lactation diet, 3 days before and after mixing around weaning) the control amount for a short period did not effectively reduce aggression and the associated stress in sows at mixing. High-Trp treatment show significant reducing the stereotype behavior (head-to-head knocking). Although, sows in the high-Trp treatment showed more total piglets born and more stillborn piglets definitely, but didn't show significant change in piglets born alive.

In 2009, E. Mosnier et al. conducted the relationship of reactivity of the sow during gestation and plasma Trp and cortisol concentrations with the feed intake during lactation. This test allowed the selection of 12 reactive (R) and 8 nonreactive (NR) sows for the study. The NR sows had decreased plasma Trp concentrations compared with the R sows during wk 1 of lactation. A low reactivity (NR) during gestation was associated with behavior of the sows that was favorable to piglet survival during farrowing, increased feed intake, and decreased plasma Trp concentration during the 1st week of lactation.

Sève et al. (1991) evaluated brain metabolites and behavior (as evaluated by subjective indication of grunts, squeals, ambulation, and exploration on 5 d, 23 d, and 45 d after weaning) in weanling pigs given varied levels (0.14, 0.23, or 0.32%) of dietary Trp. Brain Trp and Trp/LNAA concentrations were increased by dietary Trp, but they were increased similarly in emotional and non-emotional pigs. Behavioral reactivity, as determined by the results of an 'open-field' test, was not affected by dietary Trp.

Adeola and Ball (1992) found that pigs given 5g Trp/kg for five days prior to slaughter house showed significantly higher brain serotonin concentration and generally produced pork that had a lower incidence of PSE (pale, soft, and exudative). Trp supplementation lowered the incidence of PSE pork compared with pigs fed the control diet (27 vs. 33%, respectively). Adeola and Ball (1992) and Adeola et al. (1993) reported that concentrations of 5-hydroxytryptophan and serotonin in the hypothalamus of stress-

susceptible (halothane sensitive) pigs were lower than stress-tolerant pigs, even though Trp and 5-hydroxyindole acetic acid concentrations did not differ.

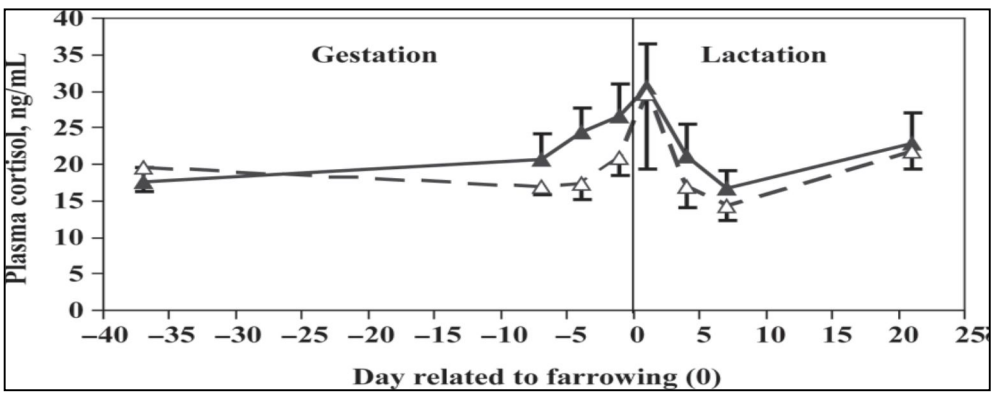
Henry et al. (1992) fed 44- to 90-kg pigs a diet deficient in Trp and reported that pigs fed the Trp-deficient diet had higher initial pH levels in the ham and loin. Dietary treatment of Trp might be the stress reducer in pig before the slaughtering. Adversely, some research suggested that dietary Trp didn't affect the pork quality (Amy, 2002; Y. Z. Li et al., 2006).

Immobilization stress increased brain levels of Trp and 5-hydroxyindoleacetic acid, a metabolite of serotonin, while it increased plasma Trp concentrations in rats (Chaouloff et al., 1989). Hayford Y et al. (2002) conducted that the impact of high temperature and dietary Trp on performance, selected organ weights and plasma free amino acid concentrations in broiler chickens. They found that performance and plasma amino acid profile deranged by heat stress are modulated, at least, to be relieved from the heat stress by feeding low Trp diet but not at all by feeding high Trp diet. However, plasma Trp/LNAA ratio is not essentially the sole factor determining resistance of chickens to heat stress.

Given the brain's need for glucose during demanding conditions, glucose intake may be beneficial for stress performance. Brain serotonin may be involved as a postprandial mechanism initiated by increases in plasma Trp to the sum of the other large neutral amino acids (Trp/LNAA ratio). Markus (2007) suggested that cold-presser stress significantly increased cortisol, reduced mood, and cognitive performance, whereas carbohydrates significantly increased plasma Trp/LNAA (30%) and positively affected performance and mood under stress. Near the parturition, mood status of sows is important for nursery performance. The newborn piglets stay in close contact with the udder to suckle colostrum and to maintain their body temperature (Rousseau et al., 1998; Orgeur et al., 2002). This requires vitality of the newborn to reach a teat, compete with its littermates, and cope with the high risk of being crushed by the sows. Quietness of the sows during parturition appears to be a component of good maternal attitude because it decreases the risk of crushing piglets, farrowing duration, and then occurrence of birth hypoxia that impairs the vitality of the newborn (Randall, 1971; Herpin et al., 1996).

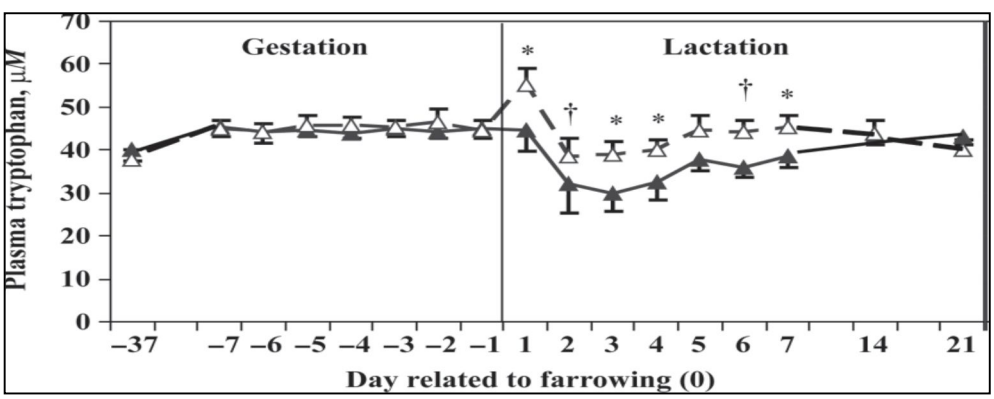
Ruis et al. (2000) observed a positive relation between the behavioral response and the increase in plasma cortisol concentration in gilts subjected to a novel environment.

Cortisol was measured in the present experiment because of the pain due to uterine contractions and piglet birth may be stressful for sows (Lawrence et al., 1994; Jarvis et al., 1998). Figures 6 and 7 showed the plasma cortisol and plasma Trp level change of the sows around the parturition.



(Adapted from E. Mosnier et al., 2009)

Figure 6. Fasted plasma cortisol concentration around parturition



(Adapted from E. Mosnier et al., 2009)

Figure 7. Fasted plasma tryptophan concentration around parturition

Consequently, applying the high level Trp in the diet has several beneficial effects such as alleviation of stress. However, as noted from these studies, there has not been enough research conducted with Trp and behavior to suggest that high dietary supplemental Trp is beneficial in reducing aggression in pigs.

4. Tryptophan on immune response to the pigs

When health status deteriorates, AAs can be used as an energy source for gluco-neogenesis, immune cell proliferation and serve as building blocks for inflammatory protein and immunoglobulin synthesis. Finally, AAs can enter specific metabolic pathways related to body defense. When the immune system is stimulated, its amino acid consumption is not always balanced by the supply and this could result in specific amino acid requirements.

Trp also plays an important role in the defense of the body and immune response regulation (Moffet and Namboodiri 2003; Le Floc'h and Seve 2007), in relation with the kynurenine pathway.

In Pigs, Melchior et al. (2004, 2005) showed that this metabolic pathway is involved in Trp metabolism disturbances associated with an inflammatory response. Pigs suffering from lung inflammation had lower plasma Trp concentrations, higher indolamine 2, 3-dioxygenase (IDO) activity in lungs and associated lymph nodes than pair-fed healthy control piglets (Le Floc'h et al., 2004, Melchior et al., 2004, 2005). Christmas et al. (2011) suggested that intestinal inflammation, malnutrition and pro-inflammatory situation may result in Trp depletion subsequently affecting the weight gain and nitrogen balance in neonatal pigs.

When disease challenged, that caused an increased demand of Trp then for the synthesis of proteins for an immune response (Le Floc'h, 2009). Dapoza (2009) also said, Trp level in the diet can be increased, especially in poor health condition. The decreases in plasma Trp concentration (higher Trp requirement) in pigs housed in poor sanitary conditions reflect the specific use of this amino acid to satisfy the immune functions of the animal (Melchior et al., 2004). Higher Trp requirement under poor sanitary condition could be explained by 2 steps.

The first one is an increase in the catabolism of Trp under the action of the IDO, which degrades Trp in the kynurenine pathway (Melchior et al., 2005). This enzyme is activated by the inflammation mediators like cytokines (such as Interferon-g). The activation of this pathway could be related to the regulation of T cells (Mellor and Munn, 2004) and/or free radical scavenger production (Christen et al., 1990) and/or specific immune related

metabolites (i.e. picolinic acid). In the mammal, Mammals possess two intracellular hemecontaining enzymes, IDO and tryptophan dioxygenase (TDO) that catalyze oxidative catabolism of Trp (Taylor, M. W., and G. Feng. 1991). Genes encoding TDO and IDO have distinct patterns of expression. In 2001, Suzuki, S. et al reported IDO is expressed at basal levels in epididymis, thymus, gut, lung, and the maternal-fetal interface during gestation and is up-regulated in response to infection and tissue inflammation. And TDO is expressed in hepatic cells and regulates homeostatic serum Trp concentrations, although TDO transcripts are present in the uterus during gestation.

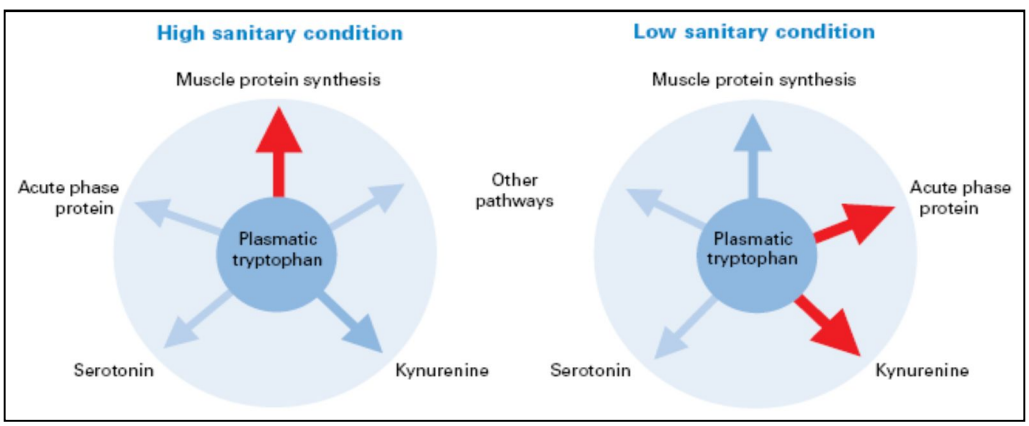
The second one is an increase in the incorporation of Trp in acute phase proteins (like haptoglobin). This acute phase proteins are Trp rich proteins compared to muscle proteins (Reeds et al, 1994). Melchior et al. (2004) reported that among piglets with inflammation, at the beginning of the experimental period, plasma haptoglobin concentration response is similar in pigs fed with the Trp deficient or Trp supplemented diets. However, at the end of the second week of the experiment, plasma haptoglobin is significantly lower in pigs fed adequate Trp diet compared with Trp deficient pigs. IDO activity in lungs is also affected by treatments. The increase in IDO activity in piglets with lung inflammation is lower in piglets fed adequate amount of Trp in diet compared to Trp deficient piglets. Lung lesions at slaughter are also less severe in L-Trp supplemented pigs.

Tejpal et al. (2008) reported that the plasma cortisol level was found to be significantly higher in the high density group than its low density counterpart. And gradual supplementation of L-Trp in diet significantly reduced the cortisol level both in high density and low density group. The continuous secretion of cortisol during chronic stress is harmful as it leads to immune suppression. Like high stocking density, constant stress response to vertebrate including fish results in the activation of the neuroendocrine system, which brings about changes in metabolism, osmoregulation, and blood parameters (Chatterjee et al., 2006; Wendelaar Bonga, 1997). The metabolism shifts from anabolism to catabolism to supply the extra energy needed to combat stress (Pickering, 1992). Much of the stress studies on fish have been focused on the primary response, i.e. cortisol and catecholamine levels (Barton and Iwama,1991a, b). Of the secondary stress response parameters, blood glucose, and lactate levels are commonly tested (Chatterjee et al., 2006). Besides, glycogen level is another indicator of the secondary responses against stress.

5. Factors for tryptophan′s metabolic pathways

Trp metabolism is involved in the response to a stressful event in weaned and growing pigs (Meunier-Salaün et al., 1991; Koopmans et al., 2005; Guzik et al., 2006). In the previous study (E. Mosnier et al., 2009), it demonstrated that quite sows had less plasma Trp concentration than the sensitive sows during the first week of lactation.

These results differ from those obtained by Sève et al. (1991) who observed that weaned piglets exhibiting a decreased reactivity had a greater plasma Trp concentration than pigs exhibiting an increased reactivity. This indicates that the relationship between reactivity and plasma Trp concentration is differs according to the age or the physiological status of the pig.



(Adapted from Ajinomoto, 2008)

Figure 8. Impacts of sanitary status on tryptophan metabolic pathways

The metabolism of growing pigs is focused toward increasing the BW under the less stressed condition, whereas the metabolism of the sows is oriented toward mobilization of body reserves (negative nutrient balance) can provide energy and nutrients for fetuses and mammary gland development and milk production.

Consequently, Trp can be metabolized in several different pathways, which are protein synthesis, kynurenine synthesis, and serotonin synthesis through the hydroxylase

pathway (Sève, 1999). However, without the information (sanitary status of pig, Figure 8), it was lack of factors or information to deciding the mainstream of Trp metabolism in the pigs.

6. Tryptophan requirements in sows

Trp is an essential amino acid while the concentration is low in plasma and contents in proteins compared with other essential AAs (Vlaardingerbroek et al., 2011). The concentration of Trp in sows is the lowest when compared with other AAs (Table 2) and it can play a rate limiting role in protein synthesis (NRC, 2012). However, S. W. Kim et al. (2013) suggested that functional AAs such as Trp may be beneficial for reducing oxidative and behavioral stress for reproductive performance with enhanced health status of sows.

Tables 3 and 4 showed the amino acid requirement in gestating and lactating sows (CVB, 2007; KSU, 2007; NRC, 2012; DPP, 2012). During the gestation, Trp requirement range (digestible Trp/Lys) in sows was huge about 15% ~ 30%. Danish nutrient standard (2015) showed the highest digestible Trp/Lys ratio, CVB (2007) was the lowest ratio. Relatively, in lactation, digestible Trp/Lys ratio was small (17% ~ 20%). The guideline sources which suggest the highest and lowest Trp/Lys ratio requirement were same.

Especially, late gestation (after d 90 after insemination) showed higher Trp/Lys ratio than early-middle gestation (until d 90 after insemination) (NRC, 2012). Trp/Lys ratio in lactation was similar or lower then gestation. However, considering the quantitative Lys requirement in the lactation feed, Trp requirement (or daily intake) higher than gestation.

Several studies reported the effects of dietary Trp level on gestating sows. Moehn et al. (2012) suggested that the Trp requirement of the 2nd parity sows increased from 1.7 to 2.6 g/d from early to late gestation. And Li et al. (2013) reported that supplementation of dietary Trp at 2.3 times (high-Trp treatment; 0.35% in the gestation diet and 0.48% in the lactation diet, 3 days before and after mixing around weaning) showed more total born piglets (12.5 vs. 10.5 piglet/litter) and more stillborn piglets (1.5 vs. 0.8 piglet/litter), but didn't show significant change in piglets born alive (10.8 vs. 9.7 piglet/litter). Mosnier et al. (2009) reported that gestating sows with low plasma Trp and cortisol concentration (nonreactive sows) increased feed intake during the 1st week of lactation (7.0 and 5.8 kg, total feed intake,

respectively, P=0.02). However, ADFI was not different between each group at 2nd and 3rd week of lactation.

Table 2. Lysine content and amino acid composoition of maternal and fetal body protein gain and of plasenta uterus, choridallantoic fluid, udder and milk expressed as percentage of lysine content

Amino acids (AAs)	**Maternal body**	**Fetal body**	**Uterus**	**Placenta + Fluid**	**Udder**	**Milk**
Lysine (g/100 CP)	6.74	4.99	6.92	6.39	6.55	7.01
AAs/Lysine x 100						
Arginine	105	113	103	101	84	69
Histidine	47	36	35	42	35	43
Isoleucine	54	50	52	52	24	56
Leucine	101	118	116	122	123	120
Lysine	100	100	100	100	100	100
Methionine	29	32	25	25	23	27
Methionine + Cysteine	45	54	50	50	51	50
Phenyalanine	55	60	63	68	63	58
Phenyalanine + tyrosine	97	102	-	-	-	115
Threonine	55	56	61	66	80	61
Tryptophan	13	19	15	19	24	18
Valine	69	73	75	83	88	71

Libal et al. (1997) showed that high dietary Trp level (22.6% Trp/Lys, 0.75% Lys) treatment showed similar results compared with control treatment (16.0% Trp/Lys, 0.75% Lys) in litter weight and litter weight gain. Lewis and Speer (1974) studied the effect of different Trp level on lactating sow. Dietary Trp level (> 0.066% in lactation feed) showed plateau effect in piglet weight gain (d 7 ~ 21 of lactation) until 0.0161% dietary Trp level

(2.10 kg and 2.38 kg, respectively). However, 0.046% dietary Trp level decreased piglet weight gain up to 32% (0.046% vs. 0.066%, 1.59 kg vs. 2.10 kg).

Table 3. Amino acids requirement in gestating sows

Items	Unit	Lys	M+C[1] Ratio	Thr[2] Ratio	Trp Ratio
NRC[3], 2012	90% DM[4], d AA[5], 3rd parity, < d 90	0.34 (% feed)	71	74	18
	90% DM, d AA, 3rd parity, > d 90	0.49 (% feed)	69	69	20
CVB[6], 2007	d AA/EW[7]	0.46 (% feed)	63	72	15
DPP[8], 2015	d AA/FU[9]	0.33 (% feed)	97	91	30
KSU[10], 2007	d AA/day	95 (g, daily)	65	75	19

[1] M+C = Methionine + Cysteine
[2] Thr = Threonine
[3] NRC = National Research Council
[4] DM = Dry matter
[5] AA = Amino acid
[6] CVB = CVB table pigs
[7] EW = Comparative energy unit in CVB (1 EW = 2,100 kcal NE)
[8] DPP = Danish nutrient standard
[9] FU = Feed unit, comparative energy unit in DPP (1 EW ≒ 2,100 kcal NE)
[10] KSU = Kansas State University

Libal et al. (2007) demonstrated that feed intake was greater for sows fed 0.17% dietary Trp (22.6% Trp/Lys) than for sows fed 0.12% Trp (16.0% Trp/Lys) at the 3rd week and overall period. Stahly et al. (1990) and Tokach et al. (1991b) have reported increases in sow reproductivity due to feeding higher crude protein diet during lactation. Roth-Maier et al. (2004) studied about Trp level (SID (standardized ileal digestible) Trp, 0.09%, 0.12%, 0.15%, 0.21%, 0.27%, 0.39%; SID Lys level, 0.88%) in lactating sows. They showed that

increasing SID Trp concentrations in the diet from 0.9 to 2.1 g/kg feed increases feed intake up to 2 times (10.0% vs. 23.8% SID Trp/Lys, respectively).

In contrast, Muns R. et al, (2010) reported total feed intake (116.36 vs. 118.43 kg, 21days after farrowing) was not affected by dietary Trp level between treatments (0.20 vs. 0.26%, total Trp level in lactation diet). In parallel as well as other phase of pig, Li et al., (2006) reported that feed intake of 2 or 4 times higher Trp supplemented treatments were not different compared with control treatment.

Table 4. Amino acids requirement in lactating sows

Items	Unit	Lys	M+C[1] Ratio	Thr[2] Ratio	Trp Ratio
NRC[3], 2012	90% DM[4], d AA[5], 1 parity	0.77 (% feed)	53	60	19
	90% DM, d AA, >2 parity	0.74 (% feed)	53	59	19
CVB[6], 2007	d AA/EW[7]	0.64 (% feed)	50	63	17
DPP[8], 2015	d AA/FU[9]	0.66 (% feed)	61	65	20
KSU[10], 2007	d AA/day	48 (g, daily)	54	63	19

[1] M+C = Methionine + Cysteine
[2] Thr = Threonine
[3] NRC = National Research Council
[4] DM = Dry matter
[5] AA = Amino acid
[6] CVB = CVB table pigs
[7] EW = Comparative energy unit in CVB (1 EW = 2,100 kcal NE)
[8] DPP = Danish nutrient standard
[9] FU = Feed unit, comparative energy unit in DPP (1 EW ≒ 2,100 kcal NE)
[10] KSU = Kansas State University

Lewis and Speer (1974) showed that dietary Trp levels (0.046, 0.066, 0.091, 0.121 and 0.161, %, dietary Trp level) did not affect total solids concentration of milk on 15 d and

d 21 of lactation (15.36, 16.06, 15.94. 15.77 and 15.98, %, mean value). In addition, Lewis and Speer (1974) indicated that dietary Trp level improve milk yield during the first 3 weeks of lactation and the effect was particularly evident at the lowest level of Trp under the restricted 5.45kg daily feed intake.

Chapter Ⅲ. Effects of Dietary Tryptophan Levels in Gestating Sows on Performance and Blood Metabolites

Abstract

This study was conducted to evaluate the dietary Trp levels in gestating sows on performance and blood metabolites. A total of 45 F1 multiparity sows (Yorkshire×Landrace) with average body weight (BW) of 218.96±2.43 kg with a parity of 4.8±0.03 was allotted to one of three treatments by parity, BW and backfat (BF) thickness in completely randomized design after artificial insemination service. Each treatment was designed based on relative ratio Trp to Lys in the gestation diet. Treatments were as followed: 1) Trp18%: 18% *ai* (apparent ileal digestible) Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. As fed basis, experimental diet contained 2,333 kcal NE/kg, 12.00% crude protein (CP), 0.501% *ai* Lys, 68% *ai* TSAA/*ai* Lys, and 72% *ai* Thr/*ai* Lys as fed basis. All other nutrients in experimental diet were met or exceeded the NRC requirement (2012). Feed intake of experimental diet at the 2nd and the 3rd parity of gestating sows was 2.2 and 2.4 kg/d, respectively. During gestation and lactation, there were no significant differences in BW, BF thickness and their changes among treatments. In farrowing performance, Trp18% treatment had higher number of total born and born alive than other treatments (P=0.07 and P=0.06, respectively). High level of Trp decreased the number of piglets in total born and born alive (linear, P=0.06). However, the number of stillborn and mummy showed no differences among treatments. Although birth weight of piglets among treatments similar, total litter weight was decreased linearly as dietary Trp level was increased (linear, P=0.07). After cross-fostering, litter weight, litter weight gain, piglet weight and piglet weight gain were not affected by dietary treatments. In addition, dietary Trp levels during gestation did not influence on average daily feed intake (ADFI) during lactation and weaning to estrus interval (WEI) of sows. However, ADFI of Trp22% and Trp26% treatments were higher than Trp18% treatment at d 10 and 11 of lactation (P<0.10). In blood metabolites, Trp22% treatment had the highest blood urea nitrogen (BUN)

concentration on d 35 of gestation (P=0.03; quadratic, P=0.01). Trp22% treatment tended to decrease blood cortisol level (P=0.07) and cortisol level showed a quadratic response to dietary Trp level on d 35 of gestation (P=0.06). High dietary Trp treatment had higher melatonin level on d 35 of gestation (P=0.02) and it showed the linear effect as dietary Trp level (P<0.01). Serotonin level showed no difference among treatments during whole experimental period. At d 35, 70, and 110 of gestation, high Trp level in gestation diet showed higher Trp concentration (linear, P<0.01; P=0.01; P<0.01, respectively) and relative Trp level to large neutral amino acids (LNAA, linear, P<0.01; P<0.01; P=0.03, respectively) of blood plasma. However, at 24 h postpartum and d 21 of lactation, there were no linear effect on Trp concentration (P=0.19; P=0.37, respectively) and relative Trp level to LNAA (P=0.60; P=0.58, respectively) although dietary Trp level for gestating sows was increased. No differences were observed in casein, fat, protein, lactose, total solid and solid not fat content of sow milk on colostrum and milk (d 21 of lactation) among treatments. Furthermore, milk production, milk dry matter (DM), and milk energy of sow milk were not affected by Trp level in gestation diet. There were no significant differences in protein, fat, and energy contents of sows and their changes during gestation and lactation. Consequently, this study demonstrated that high Trp concentration and Trp/LNAA level in blood during gestation did not show beneficial effects on farrowing performance when sows were fed diets containing *ai* Trp/ai Lys above 22%. Moreover, the piglet performances of sows were not improved in despite of high feed intake at d 10 and 11 of lactation. It represented that maximal level of *ai* Trp/*ai* Lys in gestation diet was 18% (based on 0.50% *ai* Lys in diet and 2.4 kg daily feed intake and maximal dosage of daily *ai* Trp intake may be 2.16 g during gestation.

Key words: Tryptophan, Gestating sow, Reproductive performance, Tryptophan ratio per lysine, Blood metabolites

Introduction

Tryptophan (Trp) is an essential amino acid as substrate for protein synthesis. It also serves as the precursor of serotonin and melatonin (Sainio et al., 1996). Trp has the potential to affect behaviors such as aggression and stress through its role on serotonin synthesis as well as through its role on cortisol release (Rodwell, 1979; Lepage et al., 2002).

Generally, gestating sows were raised in stall under the restricted feeding scheme for productivity. These stressful environments had negative effect on gestating sows and its progeny. However, these stresses could be reduced by Trp supplementation as Trp had positive effects on reducing the stress response through secretion of melatonin and serotonin (Poletto et al., 2010; Li et al., 2011; Shen et al., 2015). Trp induced the melatonin secretion, which blocks adrenocorticotropin (Rose and Sterner, 1992; Rose, 1995) and serotonin secretion, which blocks cortisol directly (Lepage et al., 2012). Poletto et al. (2010) demonstrated that additional Trp reduced the behavioral activity and aggressive action of grower gilts. In addition, Li et al. (2006) reported that increasing dietary Trp reduced the total fighting time by approximately 50% after re-grouped grower-finisher pigs. These Trp effects could reduced parturition stress in gestating sows, so it decreases the risk of crushing piglets, farrowing duration, and then occurrence of birth hypoxia that impairs the vitality of the newborn (Randall, 1971; Herpin et al., 1996).

Additional Trp supplementation improved growth performance and protein deposition on pigs (Meunier-Slaun et al., 1991; Henry, 1995; Koopmans et al., 2005). Since Trp is an essential component of body muscle tissue (Sidransky, 1985), increasing dietary Trp level increased protein synthesis in muscle and improved growth performance in pig. Because of these functions, supplementation of Trp was expected to increase fetal growth rate and improve maternal body condition. It is important that healthy fetus and good body condition of sow are critical factors to improving productivity and longevity of sows.

Therefore, the present study was conducted to estimate the effect of increasing dietary Trp level on the reproductivity of gestating sows under restricted feeding and stall housing. Moreover it was expected that optimum Trp requirement for gestating sows can be determined for improve reproductive performance.

Materials and Methods

Animal and housing

A total of 45 F1 multiparity sows (Yorkshire×Landrace) with average BW of 218.96±2.43 kg and a parity of 4.8±0.03 was allotted to one of three treatments by parity, BW and back-fat thickness in completely randomized design after two times of artificial insemination service. All sows were checked the estrus return on d 21 and confirmed pregnancy at d 35 of gestation by ultrasound scanner (Dongjin BLS, Korea).

All sows were fed 2.4 kg/d of experimental diet (the 2nd parity sows fed 2.2 kg) and fed commercial lactation diet restrictively during 5 days postpartum (increase 1 kg/d) then lactating sows were fed diet *ad libitum*. Within 24 h postpartum, Fe-dextran (150 ppm) injection, ear notching, needle teeth clipping and tail docking were practiced to all piglets. Furthermore, male piglets were castrated in d 3 postpartum. Piglets were cross-fostered across treatments within 1 day after birth to balance suckling intensity of sows with equalization of litter size, and thus to minimize any affect of initial litter size potentially affecting litter growth.

All sows were accommodated in individual gestation stalls (2.40m×0.64m) where the indoor temperature was regulated by automatic ventilation system (average 19℃, ranged 17 - 21℃). At d 110 of gestation, sows were moved from gestation barn to farrowing crates (2.20m×0.65m) with partition walls (2.50m×1.80m) after washing and disinfecting their body. During lactation, the room temperature and air condition of farrowing barn were kept automatically at 28±3℃ by heating lamps and ventilation fans and air-conditioner in farrowing barn. After weaning, sows were moved to breeding barn again for the next conception.

Treatment and experimental diet

Each treatment was designed based on relative ratio Trp to Lys in the gestation diet. Treatments were as followed: 1) Trp18%: 18% *ai* (apparent ileal digestible) Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. An experimental diet (gestating sows' diet) contained 2,333 kcal NE/kg, 12.00% crude protein,

0.501% *ai* Lys, 68% *ai* TSAA/*ai* Lys, and 72% *ai* Thr/*ai* Lys as fed basis. All nutrients were met or exceeded NRC (2012) nutrient requirement. The formula and chemical composition of experimental diets by each treatment were presented in Table 5.

Measurements

Body weight and backfat thickness of sows were measured at d 0, 35, and 110 of gestation, 24 h postpartum, and d 21 of lactation, respectively. An ultra-sound device (Lean meter, Renco Corp., Minneapolis, USA) was used for measuring backfat (BF) thickness at P_2 position (mean value from both side of the last rib and 65 mm away from the backbone). The numbers of total born and born alive were recorded within 24 h postpartum and the number of piglets was recorded after cross-fostering and at d 21 of lactation. Piglet weight was recorded before cross-fostering and at d 21 of lactation. Total litter weight was summed from piglet alive, stillborn, mummy and alive litter weight was only from summing the live piglets weight. Litter weight was calculated by summing the individual piglet weights. Feed intake was recorded daily during experimental period. Weaning to estrus interval (WEI) was determined by monitoring for estrus from after weaning.

Blood samples of sows were collected from the anterior vena cava of 5 sows per treatment at d 0, d 35, d 70, d 110 of gestation and 24 h postpartum as well as d 21 of lactation. Blood samples of piglets were also collected from the anterior vena cava of 5 piglets at 24 h postpartum and d 21 of lactation from each treatment (1 piglet blood sampling from 1 litter of sow and total 5 piglets were selected from different 5 in same treatment). All samples were enclosed into collecting tube (serum and EDTA tube, BD Vacutainer®) and centrifuged at 3,000 rpm and 4℃ for 5 minutes after clotting at room temperature for 30 minutes. The upper liquid (serum) of the blood was separated to a microtube (Axygen, Union City, CA, USA) and stored at -20℃ until later analysis. Cortisol and melatonin were analyzed by ELISA kit (Endocrine technologies, USA; IBL, Germany) and serotonin was analyzed by HPLC (Recipe, Germany). The concentration of blood urea nitrogen (BUN) and glucose were analyzed by kinetic assay (Modular analytics, Roche, Germany) and free fatty acid (FFA) and total protein were analyzed by colorimetry (Moldular analytics, Hitachi, Japan). Amino acid was analyzed by LC-MS/MS method (LC-MS/MS, 3200 Q TRAP, AB

SCIEX, USA).

Colostrum and milk samples were taken 5 sows of each treatment at 24 h postpartum and d 21 of lactation, respectively. After injection of oxytocin (Komioxytocin inj., Komipharm international Co. Ltd., Siheung, Korea) 0.3ml (10IU/ml), milks were collected from first or second teat of sow. After milk collection in conical tube (50ml), samples were stored in a freezer (-20℃) until further analysis. Proximate analysis of colostrum and milk was conducted using Milkoscan FT 120 (FOSS Electric, Sungnam, Korea). The milk production (g), milk dry matter (g), and milk energy (kcal) were calculated from piglet weights and growth rates by equations in Noblet and Etienne (1989). The prediction of fat (kg), energy (MJ), and protein (kg) contents of sows (gestation d 0 and 110; lactation d 0 and 21) was from empty body weight (EBW=0.96*BW, kg) and backfat thickness (P_2 site, mm) by the equation of Dourmad et al. (2008).

Statistical analysis

All statistical analyses by least squares mean comparisons were carry out using the PDIFF option with the General Linear Model procedure of SAS (SAS Institute, 2004). Orthogonal polynomial contrasts were used to determine linear and quadratic effects by increasing Trp levels in lactation for all measurements of sows and piglets, blood profile, and milk composition. Individual sows were used as the experimental unit in performance and each sample was used as the experimental unit in blood and milk analysis. Probability values less than 0.05 ($P<0.05$) were considered as significant difference; $0.05 \leq P < 0.10$ were indicative of a trend; and values equal to or greater than 0.10 were considered as non-significant difference.

Results and Discussion

Reproductive and litter performance

Effects of dietary Trp levels in gestating sows on reproductive performance and their progeny growth performance were presented in Table 6. In reproductive performance, Trp18% treatment had higher piglet number of total born and born alive than other

treatments (P=0.07 and P=0.06, respectively). In addition, high level of Trp decreased the number of piglets in total born and born alive (linear, P=0.06). However, the number of stillborn and mummy showed no differences significantly among treatments. Although birth weight of piglets among treatments was similar, total litter weight was decreased linearly as dietary Trp level was increased (linear, P=0.07). After cross-fostering, litter weight, litter weight gain, piglet weight, and piglet weight gain were not different among treatments.

Several studies reported the effects of dietary Trp level on gestating sows. Moehn et al. (2012) suggested that the Trp requirement of the 2nd parity sows increased from 1.7 to 2.6 g/d from early to late gestation. And Li et al. (2013) reported that supplementation of dietary Trp at 2.3 times (high-Trp treatment; 0.35% in the gestation diet and 0.48% in the lactation diet, 3 days before and after mixing around weaning) showed more total born piglets (12.5 vs. 10.5 piglet/litter) and more stillborn piglets (1.5 vs. 0.8 piglet/litter), but didn't show significant change in piglets born alive (10.8 vs. 9.7 piglet/litter). Until d 3 of gestation, Trp level of the present study was from 3.07 g/d (Trp18% treatment) to 4.08 g/d (Trp26% treatment) that was 1.6 to 2.4 times higher than suggested Trp level by Moehn et al. (2012). Nevertheless Trp22% and Trp26% treatments showed lower number of total born and born alive than Trp18% treatment (Table 6), this result was disagree with previous studies. Reproductive performance may not be greatly affected by dietary Trp level but it might be affected by individual sows' condition such as artificial insemination service, ovulation rate, conception rate and optimal mating time (Soede, 1995; Kemp 1996; Knox et al., 2002). In conclusion, over the 18% *ai* Trp/*ai* Lys (0.50% *ai* Lys) in gestation diet may not improve farrowing and nursery performance. More studies are needed to demonstrate effect of dietary Trp level on gestating sows, clearly.

In addition, dietary Trp levels during gestation did not affect average daily feed intake (ADFI) and WEI during lactation. But, ADFI of Trp22% and Trp26% treatments were higher than Trp18% treatment at d 10 and 11 of lactation (Figure 9). A rapid increase in daily feed intake is important to lactating sow (Noblet et al., 1990) and insufficient feed intake during lactating results in greater BW loss, decreased milk production and reproductive problems that may lead to culling of the sow (Baidoo et al., 1992; Eissen et al., 2000). Mosnier et al. (2009) reported that gestating sows with low plasma Trp and cortisol concentration (nonreactive sows) increased feed intake during the 1st week of lactation (7.0

and 5.8 kg, total feed intake, respectively, P=0.02). However, ADFI was not different between each group at 2nd and 3rd week of lactation. Trp plays a vital role in synthesis of neurotransmitter serotonin. Serotonin in turns directly blocks cortisol in rainbow trout (Lepage et al., 2002), which is similar with current study. However, additional studies are needed to prove for this effect.

Low dietary Trp levels (0.64 vs. 1.84 g/d) in gestation delayed the days of WEI (37.0 and 6.1 days, respectively) and result in poor body conditions after weaning (Meisinger and Speer, 1979). Poor body condition after lactation had negative impact on WEI (Vesseur et al., 1994) and eventually negative impact on reproductivity of following parity. Regardless of daily Trp intake 3.07 (Trp18% treatment) to 4.08 g/d (Trp26% treatment), WEI was not improved when gestating sows were fed high Trp diet (> 3.07 Trp g/d).

Body weight and backfat thickness

Effects of dietary Trp levels of gestating sows on BW and BF thickness during gestating and lactating period were presented in Table 7. During gestation and lactation, there were no significant differences in BW, BF thickness, and their changes. Trp18 treatment showed heavier BW (253 kg) at d 110 of gestation than Trp 22% and Trp26% treatments (240 and 243 kg, respectively) numerically, which can perfectly explain by higher number of total born piglet of Trp18% treatment than other treatments (up to 4.0 - 4.2 piglets).

Meisinger and Speer (1979) reported that BW gain of sow was increased linearly during gestation and no difference during lactation as dietary Trp increase from 0.64 g/d to 2.24 g/d. In addition, BW changes of lactating sow were not significantly affected by dietary treatments during gestation. In this study, presumably total energy and AAs intake among treatments were similar, as gestating sows were fed restricted and ADFI during lactation was not different among treatments. In addition, daily intake Trp levels of this study (3.07 to 4.08g/d) may be exceeded the highest level of the previous studies (2.24 g/d (Meisinger and Speer, 1979); 2.6 g/d (Moehn et al., 2012)). In conclusion, over 18% *ai* Trp/*ai* Lys (0.50% *ai* Lys) in gestation diet exceeded optimal requirement and did not affect BW and BF changes of sows.

Blood metabolites

The effect of dietary Trp levels in gestating sows on blood metabolites during gestating and lactating period was presented in Table 8. Trp22% treatment had the highest blood BUN concentration on d 35 of gestation (quadratic, P=0.01). Trp22% treatment tended to decrease blood cortisol level (P=0.07) and dietary Trp levels showed quadratic response in cortisol level on d 35 of gestation (quadratic, P=0.06). High Trp treatment had higher melatonin level on d 35 of gestation (P=0.02) and it was also increased linearly as dietary Trp level increased (linear, P<0.01). Serotonin level showed no difference during whole experimental period among treatments.

Munchow and Bergner (1968) reported that a negative correlation between the biological value of feed and BUN content (correlation coefficient 0.96 for pigs, with egg protein as a reference protein). Excessive consumption of protein or amino acid decreased the availability of protein (Jeong et al., 2010) and increased the excretion of the nitrogen as urea form (Han et al., 2001). Thus, increase of BUN concentration indicated that excessive AAs are inefficiently metabolized and circulated in the blood before excretion (Jeong et al., 2010). These previous findings were in agreement with the present study. This study had effect on BUN of d 35 gestating sows, although there was no significant difference in BUN at d 70 and d 110 gestating sows (Table 8). Regardless of sow parity, protein deposition was greater in late than early gestation and fetal growth associate with more to amino acid requirements in late gestation (Soenke et al., 2012). The present study shows that the ratio of *ai* Trp/*ai* Lys (0.50% *ai* Lys) above 18% could be excessive than requirement during early gestation.

Poletto et al. (2010) reported the effect of additional Trp on the behavior and aggressive in grower gilt. They found that higher level of Trp in the diet reduced the behavioral activity and the aggressive action of grower gilts with increasing plasma serotonin concentration (up to 12.8 to 20.3%). Most likely this was due to the increased production of serotonin. Trp plays a vital role in synthesis of neurotransmitter serotonin. Serotonin in turns directly blocks cortisol in rainbow trout (Lepage et al., 2002). Trp is not only metabolized to serotonin, but subsequent metabolism of serotonin results in the formation of melatonin. Previous findings were similar with the results of present experiment that high Trp treatment showed higher melatonin level on d 35 of gestation. It represented,

from what has been said above, that the level of *ai* Trp/*ai* Lys (0.50% *ai* Lys) above 18% could be excessive during early gestation. Although excessive dietary Trp level may lead to decrease the availability of protein during early gestation, it showed reduction of cortisol with high blood melatonin concentration.

Amino acid profiles in blood

Effect of Trp supplementation level on amino acid profiles of gestating and lactating sows were presented in Tables 9, 10 and 11, respectively. At d 35, 70, and 110 of gestation, increased Trp level in gestation diet resulted in the higher Trp concentration (linear, $P<0.01$; $P=0.01$; $P<0.01$, respectively) and relative Trp level to LNAA (linear, $P<0.01$; $P<0.01$; $P=0.03$, respectively) of blood plasma. However, at 24 h postpartum and d 21 of lactation, there were no linear effect on Trp concentration (linear, $P=0.19$; $P=0.37$, respectively) and relative Trp level to LNAA (linear, $P=0.60$; $P=0.58$, respectively) as Trp level of gestation diet was increased. This result indicated that dietary Trp level in gestation sows affected on the plasma Trp concentration and Trp/LNAA ratio during gestation not lactation.

Several studies were conducted to demonstrate the effect of dietary Trp level on blood Trp and LNAA concentration. Meisinger and Speer (1979) reported that plasma Trp concentration was increased from 0.40 mg/100ml to 2.20 mg/100ml (5.5 times) as dietary Trp increased from 0.64 g/d to 2.24 g/d (3.5 times). Moreover plasma LNAA was changed from 13.5 mg/100ml to 24.2 mg/100ml (1.79 times) in gestating sows. Libal et al. (1997) showed the dietary Trp level (0.12% vs. 0.17%, 0.75% Lys in lactation feed) affected the plasma Trp, Phe, and Val concentrations (0.26 vs. 1.18, 0.75 vs. 1.25, and 1.60 vs. 2.23, mg/dl, respectively). In the present study, plasma Trp concentration and Trp/LNAA ratio were different among treatments (Table 10), which is in agreement with the previous findings. These results suggest that dietary Trp level increased plasma concentration of Trp and LNAA, however increasing rate of plasma Trp was higher than LNAA, which resulted in changing Trp/LNAA ratio. Conversely, Lewis and Speer (1974) reported that some LNAA (Ile, Phe, and Val) concentration of plasma reduced with maintained plasma Trp by high dietary Trp level. Additional experiments are needed to demonstrate relationship between dietary Trp and plasma AAs concentration.

Milk composition and estimated nutrient output

Effects of dietary Trp levels in gestating sows on milk composition and estimated nutrients output of milk were presented in Table 12. No differences were observed in casein, fat, protein, lactose, total solid and solid not fat content of sow milk on colostrum and milk (d 21 of lactation) among treatments. Furthermore, milk production, milk dry matter (DM), and milk energy were not affected by dietary Trp level during gestation.

After low energy supply during gestation, body reserve mobilization for milk production in sows cannot be compensated by higher energy supply during lactation (Beyer et al. 2007), which in accordance with result of current study. The present study (Table 7) showed the BW and BF thickness were not different during gestation and lactation. Additionally, Beyga and Rekiel (2010) suggested that high BF sows at late gestation (26.2 vs. 15.2, BF thickness on d 104 of gestation) showed better litter performance and rearing of piglets with similar ADFI during lactation (4.98 kg/d vs. 5.09 kg/d). In summary, dietary level of *ai* Trp/*ai* Lys (0.50% *ai* Lys in gestation feed) above 18% did not affect the body reserve mobilization for milk production during gestation.

Protein, fat, and energy contents and their deposition

Effects of dietary Trp levels in gestating sows on protein, fat, and energy contents and their deposition during gestating and lactating period were presented in Table 13. Meisinger and Speer (1979) show that increasing dietary Trp resulted in significant linear increases in daily nitrogen retention. When sows fed various Trp diets (1.04, 1.44, 1.84 and 2.24, Trp g/d), they showed the plateau trend (>1.84 g/d) of nitrogen retention (2.5, 3.7, 5.0, and 5.5, g, respectively). Supplemented Trp levels of the present study (3.07 ~ 4.08 Trp g/d) exceeded the highest level of the previous finding, which treatments may not affect the nitrogen retention among treatments.

Moreover, Moehn et al. (2011) suggested that restricted and phase feeding during gestation can control body condition of sows. That means that energy intake is the limiting factor for fat deposition and fat mobilization. Presumably energy and AAs intake among treatments were similar among treatments, because sows were fed restricted during gestation and ADFI during lactation was not different among treatments. Because energy level of gestation diet of each treatment was same (2,333 NE kcal/kg) and sows were fed commercial

diet during lactation regardless of treatments. Consequently, dietary level of Trp on gestating sows had no effect on deposition of protein, fat, and energy in the body. Additional studies are needed to demonstrate the effect of dietary Trp on body composition of sows.

Conclusion

During gestation and lactation, there were no significant differences in BW, BF thickness and their changes among treatments. After cross-fostering, litter weight, litter weight gain, piglet weight and piglet weight gain were not different among treatments. In addition, dietary Trp levels in gestating sows did not affect ADFI and WEI during lactation. No differences were observed in milk composition, milk production, milk DM, and milk energy were not affected by dietary Trp level. Also, there were no significant differences in protein, fat, and energy contents of sows and their changes during gestation and lactation.

In conclusion, this experiment demonstrated that high Trp concentration and Trp/LNAA level in blood during gestation did not show beneficial effects on farrowing performance when sows were fed diets containing *ai* Trp/*ai* Lys above 22%. Moreover, the nursery performances of sows were not improved despite of high feed intake at d 10 and 11 of lactation. It represented that maximal level of *ai* Trp/*ai* Lys in gestation diet was 18% (based on 0.50% *ai* Lys in diet and 2.2 ~ 2.4 kg daily feed intake). In particular, maximal dosage of daily *ai* Trp intake may be 1.98 ~ 2.16 g during gestation. Further studies would be needed to demonstrate the effects of lower Trp level in gestation diet (0.50% *ai* Lys) than 18% *ai* Trp/*ai* Lys on sows.

Table 5. Formula and chemical composition of experimental diet in gestation

Items	Treatment[1]		
	Trp18%	Trp22%	Trp26%
Ingredient, % (as fed basis)			
Corn	49.42	49.02	48.62
Wheat	20.00	20.00	20.00
Soybean meal-45%CP	5.56	5.56	5.56
Rapeseed meal	4.00	4.00	4.00
Wheat bran	7.32	7.40	7.46
Soy hull	6.50	6.50	6.50
Mixed animal fat	1.08	1.20	1.30
Molasses	3.00	3.00	3.00
L-lysine·HCl	0.52	0.52	0.52
L-tryptophan 10%[2]	0.00	0.21	0.42
L-threonine 99%	0.08	0.08	0.08
Mono-di calcium phosphate	0.46	0.46	0.46
Limestone	1.21	1.20	1.21
Salt	0.35	0.35	0.35
Vitamin and mineral mix[3]	0.43	0.43	0.43
Phytase (1,000 IU)	0.07	0.07	0.07
Chemical composition (calculated values, as fed basis)			
NE, Kcal/kg	2,333	2,333	2,333
Crude protein, %	12.0	12.0	12.0
Total Lys, %	0.638	0.638	0.637
Total Trp, %	0.128	0.149	0.170
ai Lys, %	0.501	0.501	0.501
ai Trp, %	0.089	0.110	0.130
ai TSAA / *ai* Lys, %	68	68	68
ai Thr / *ai* Lys, %	72	72	72
ai Trp / *ai* Lys, %	18	22	26
Ca, %	0.7	0.7	0.7
Total P, %	0.4	0.4	0.4

[1] Trp18% (*ai* Trp/*ai* Lys, 18%), Trp22% (*ai* Trp/*ai* Lys, 22%), and Trp26% (*ai* Trp/*ai* Lys, 26%).
[2] Feed grade (CJ, Indonesia).
[3] Supplied the following per kilogram of diet: vitamin A, 15,020 IU; vitamin D_3, 3,000 IU; choline chloride, 1,200 mg; vitamin E, 80 IU; cyanocobalamine, 40 mg; niacin, 20.0 mg; Ca panthotenate, 16.0 mg; riboflavin, 4.0 mg; menadione, 4.0 mg; folic acid, 2 mg; pyridoxine, 2 mg; thiamine 0.8 mg; d-biotin, 0.5 mg; Fe, 200.0 mg (as $FeSO_4$); Mn, 79.0 mg (as $MnSO_4$); Zn, 35.0 mg (as $ZnSO_4$); Cu, 5.0 mg (as $CuSO_4 5H_2O$); Co, 1.98 mg (as $CoSO_4$); I, 1.26 mg (as $Ca(IO_3)$); Se, 0.1 mg (as Na_2SeO_3).

Table 6. Dietary tryptophan levels in gestation on farrowing, their progeny growth performance, average daily feed intake and weaning to estrus interval in sows

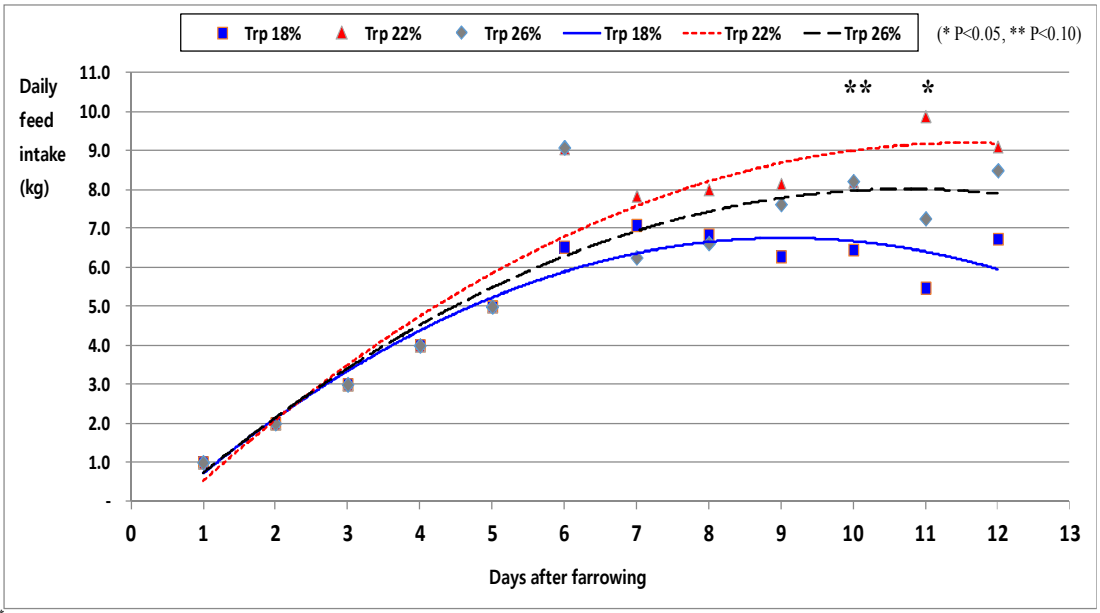
Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
No. of piglets							
Total born	16.2	11.8	12.2	0.752	0.07	0.06	0.16
Stillborn	1.2	0.6	0.7	0.177	0.54	0.33	0.56
Mummy	0.4	0.3	0.0	0.089	0.32	0.15	0.72
Born alive	14.6	10.9	11.5	0.632	0.06	0.06	0.11
After cross-fostering	---------- 11.1 ----------						
d 21 of lactation	9.6	10.4	10.7	0.226	0.36	0.18	0.63
Litter weight, kg							
Total litter weight	20.36	16.16	16.42	0.82	0.11	0.07	0.22
Alive litter weight	18.75	15.50	15.83	0.72	0.16	0.11	0.25
24 h postpartum	15.22	16.30	16.30	0.468	0.58	0.39	0.58
d 21 of lactation	65.24	67.91	70.64	1.443	0.31	0.13	0.89
Gain (d 0 - 21)	50.02	51.61	54.34	1.231	0.35	0.16	0.74
Piglet weight, kg							
24 h postpartum	1.32	1.48	1.44	0.040	0.30	0.25	0.29
d 21 of lactation	6.27	6.59	6.52	0.113	0.46	0.25	0.65
Gain (d 0 - 21)	4.95	5.11	5.08	0.101	0.68	0.39	0.92
Average daily feed intake, kg/d							
	5.49	5.37	6.72	0.31	0.23	0.18	0.33
Weaning to estrus interval, d							
	3.83	4.17	4.11	0.07	0.08	0.06	0.12

[1] A total of 35 sows (Yorkshire×Landrace) with Trp18%:10 sows, Trp22%:11 sows, Trp26%: 14 sows.
[2] Standard error of mean.

Table 7. Effects of dietary tryptophan levels in gestation on body weight and backfat thickness in sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Body weight, kg							
Gestation							
Initial (d 0)	220.5	219.4	218.3	2.47	0.92	0.68	1.00
d 35	227.6	223.6	223.5	2.68	0.72	0.48	0.70
d 110	253.4	240.4	242.7	4.11	0.41	0.38	0.29
Gain (d 0 - 110)	33.2	21.0	24.4	2.75	0.43	0.25	0.51
Lactation							
24 h postpartum	233.5	224.7	226.5	3.70	0.72	0.47	0.70
d 21	223.0	215.9	217.1	3.52	0.76	0.51	0.74
Changes (d 0 - 21)	-10.5	-8.8	-9.4	1.65	0.96	0.82	0.86
Backfat thickness, mm							
Gestation							
Initial (d 0)	18.8	18.1	18.3	0.73	0.93	0.78	0.81
d 35	20.8	19.6	21.1	0.71	0.55	0.82	0.29
d 110	22.9	20.9	22.1	0.89	0.54	0.42	0.42
Gain (d 0 - 110)	4.1	2.8	3.8	0.72	0.66	0.39	0.75
Lactation							
24 h postpartum	20.7	19.5	21.9	0.92	0.45	0.39	0.38
d 21	19.1	18.6	20.4	0.82	0.50	0.35	0.51
Changes (d 0 - 21)	-1.6	-0.9	-1.5	0.47	0.81	0.78	0.57

[1] A total of 45 sows (Yorkshire×Landrace) with average body weight of 218.96±2.43 kg and a parity of 4.8±0.03.
[2] Standard error of mean.



* A total of 35 sows (Yorkshire×Landrace) with Trp18%:10 sows, Trp22%:11 sows, Trp26%: 14 sows.

Figure 9. Effect of dietary tryptophan levels in gestation on daily feed intake during early lactation

Table 8. Dietary tryptophan levels in gestation on blood metabolites in sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
BLOOD UREA NITROGEN, mg/dL							
Gestation							
Initial (d 0)	---------- 13.82 ----------						
d 35	9.98[b]	12.38[a]	10.45[ab]	0.498	0.03	0.96	0.01
d 70	11.26	11.80	12.54	0.500	0.36	0.22	0.50
d 110	9.94	9.00	9.46	0.359	0.50	0.57	0.32
Lactation							
24 h postpartum	10.46	12.50	11.38	0.455	0.22	0.23	0.19
d 21	16.66	12.42	20.25	0.951	0.06	0.08	0.07
Cortisol, ng/mL							
Gestation							
Initial (d 0)	---------- 2.55 ----------						
d 35	5.15	3.73	4.63	0.542	0.07	0.10	0.06
d 70	2.48	2.48	2.23	0.322	0.94	0.75	0.98
d 110	3.15	3.02	2.37	0.323	0.86	0.61	0.89
Lactation							
24 h postpartum	6.67	5.99	4.20	1.072	0.82	0.63	0.76
d 21	2.27	3.88	2.31	0.471	0.75	0.92	0.18
Melatonin, pg/mL							
Gestation							
Initial (d 0)	---------- 61.98 ----------						
d 35	92.45[b]	108.10[ab]	121.25[a]	4.583	0.02	<0.01	0.91
d 70	92.10	110.20	124.65	11.647	0.57	0.31	0.92
d 110	51.28	70.58	50.73	6.770	0.60	0.98	0.33
Lactation							
24 h postpartum	53.36	47.76	54.62	4.824	0.57	0.59	0.38
d 21	251.34	196.98	301.40	19.252	0.14	0.36	0.08
Serotonin, ng/mL							
Gestation							
Initial (d 0)	---------- 218.2 ----------						
d 35	522.0	354.6	542.1	57.72	0.31	0.83	0.14
d 70	471.3	238.6	432.4	57.16	0.30	0.80	0.14
d 110	469.5	216.2	420.0	53.74	0.24	0.71	0.11
Lactation							
24 h postpartum	297.0	185.8	351.1	28.478	0.08	0.40	0.04
d 21	318.8	167.0	294.2	26.529	0.08	0.42	0.04

[1] Blood samples from 4 sows per treatment.
[2] Standard error of mean.
[abc] means with different superscripts within the same row significantly differ ($P<0.05$).

Table 9. Effect of dietary tryptophan levels in gestation on amino acid profiles in blood plasma of gestating sows (cont')

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Arginine, μmol/L							
Initial (d 0)	---------- 193.0 ----------						
d 35	243.6	232.8	218.2	10.04	0.67	0.39	0.94
d 70	252.8	274.5	269.0	8.65	0.73	0.52	0.66
d 110	174.8	192.8	191.2	9.05	0.44	0.41	0.33
Histidine, μmol/L							
Initial (d 0)	---------- 80.0 ----------						
d 35	82.2^{b}	96.2^{a}	96.4^{a}	2.80	0.04	0.02	0.16
d 70	105.6	120.5	120.8	4.34	0.34	0.18	0.55
d 110	100.4	115.0	106.4	3.05	0.13	0.36	0.07
Isoleucine, μmol/L							
Initial (d 0)	---------- 131.0 ----------						
d 35	116.2	127.8	113.8	3.27	0.26	0.78	0.11
d 70	148.6	159.0	153.0	3.03	0.28	0.56	0.15
d 110	129.2^{b}	149.0^{a}	154.6^{a}	5.35	0.02	0.01	0.17
Leucine, μmol/L							
Initial (d 0)	---------- 243.0 ----------						
d 35	237.6	253.0	229.8	5.15	0.23	0.55	0.11
d 70	271.8	289.5	273.2	5.69	0.32	0.92	0.14
d 110	187.2^{b}	226.8^{a}	230.6^{a}	8.07	0.04	0.02	0.28
Lysine, μmol/L							
Initial (d 0)	---------- 364.0 ----------						
d 35	304.6	358.4	287.0	16.01	0.23	0.67	0.10
d 70	347.0^{b}	395.5^{a}	340.0^{b}	12.90	0.04	0.76	0.02
d 110	173.0^{b}	267.3^{a}	240.6^{ab}	16.74	0.05	0.08	0.06
Methionine, μmol/L							
Initial (d 0)	---------- 65.0 ----------						
d 35	76.6	76.4	75.4	3.36	0.99	0.90	0.96
d 70	99.8	101.0	103.6	2.17	0.78	0.54	0.75
d 110	73.0^{b}	91.3^{a}	93.8^{a}	3.90	0.02	0.01	0.09

[1] Blood samples from 4 sows per treatment.
[2] Standard error of mean.
[abc] means with different superscripts within the same row significantly differ (P<0.05).

Table 10. Effect of dietary tryptophan levels in gestation on amino acid profiles in blood plasma of gestating sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Phenylalanine, μmol/L							
Initial (d 0)	---------- 104.0 ----------						
d 35	98.8	104.8	102.4	2.05	0.59	0.55	0.42
d 70	105.2	105.8	111.4	2.70	0.55	0.32	0.69
d 110	80.6	91.0	93.6	3.12	0.12	0.06	0.40
Threonine, μmol/L							
Initial (d 0)	---------- 210.0 ----------						
d 35	197.0^{b}	246.2^{a}	213.2^{ab}	7.12	<0.01	0.20	<0.01
d 70	241.0	261.5	254.6	6.29	0.42	0.37	0.34
d 110	167.6^{b}	180.3^{ab}	214.0^{a}	9.44	0.06	0.02	0.81
Tryptophan, μmol/L							
Initial (d 0)	---------- 82.0 ----------						
d 35	74.2^{b}	86.6^{ab}	90.6^{a}	2.19	<0.01	<0.01	0.08
d 70	78.2^{b}	85.5^{ab}	89.8^{a}	1.82	0.03	0.01	0.57
d 110	57.4^{b}	72.3^{a}	76.6^{a}	2.68	<0.01	<0.01	0.04
Tyrosine, μmol/L							
Initial (d 0)	---------- 135.0 ----------						
d 35	94.8^{b}	112.8^{a}	109.8^{ab}	3.11	0.01	0.02	0.04
d 70	118.8	120.8	119.0	2.68	0.62	0.97	0.34
d 110	92.8	100.5	103.2	3.50	0.48	0.29	0.60
Valine, μmol/L							
Initial (d 0)	---------- 359.0 ----------						
d 35	313.6	347.6	307.4	7.41	0.11	0.73	0.04
d 70	367.4	403.5	379.8	11.17	0.20	0.60	0.09
d 110	235.2^{b}	308.3^{a}	298.0^{a}	13.51	0.04	0.03	0.12
Trp/LNAA[3] ratio							
Initial (d 0)	---------- 0.0845 ----------						
d 35	0.0863^{b}	0.0920^{b}	0.1050^{a}	0.0026	<0.01	<0.01	0.30
d 70	0.0779^{b}	0.0793^{b}	0.0867^{a}	0.0016	0.01	<0.01	0.97
d 110	0.0792^{b}	0.0832^{a}	0.0876^{a}	0.0018	0.07	0.03	0.78

[1] Blood samples from 4 sows per treatment.
[2] Standard error of mean.
[3] Large neutral amino acid (sum of Ile, Leu, Val, Phe, and Tyr).
[abc] means with different superscripts within the same row significantly differ (P<0.05).

Table 11. Effect of dietary tryptophan levels in gestation on amino acid profiles in blood plasma of lactating sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Arginine, μmol/L							
24 h postpartum	230.0	209.6	240.5	18.40	0.21	0.26	0.17
d 21 of lactation	213.2^{ab}	166.2^{b}	228.4^{a}	11.77	0.07	0.54	0.03
Histidine, μmol/L							
24 h postpartum	118.6	116.6	132.5	5.13	0.21	0.15	0.28
d 21 of lactation	102.4	101.0	98.2	3.64	0.92	0.70	0.94
Isoleucine, μmol/L							
24 h postpartum	131.0	120.6	130.2	7.90	0.52	0.58	0.33
d 21 of lactation	113.0^{ab}	95.2^{b}	138.2^{a}	7.05	0.04	0.12	0.04
Leucine, μmol/L							
24 h postpartum	180.2	191.4	203.8	11.69	0.26	0.12	0.71
d 21 of lactation	181.2^{ab}	144.4^{b}	211.6^{a}	11.18	0.05	0.21	0.03
Lysine, μmol/L							
24 h postpartum	294.4	305.4	315.5	29.94	0.77	0.51	0.84
d 21 of lactation	209.6^{a}	148.8^{b}	211.8^{a}	12.71	0.07	0.94	0.03
Methionine, μmol/L							
24 h postpartum	106.8	106.6	114.3	6.51	0.35	0.22	0.45
d 21 of lactation	45.4	36.4	43.2	2.72	0.46	0.77	0.24
Phenylalanine, μmol/L							
24 h postpartum	90.0	84.0	87.8	3.3.	0.52	0.88	0.27
d 21 of lactation	87.0	76.4	90.2	3.9	0.21	0.67	0.09
Threonine, μmol/L							
24 h postpartum	294.8	292.0	320.8	19.3	0.50	0.33	0.52
d 21 of lactation	188.0	133.4	191.0	13.1	0.09	0.91	0.03
Tryptophan, μmol/L							
24 h postpartum	67.6	66.6	78.8	4.2	0.28	0.19	0.37
d 21 of lactation	54.8	54.2	61.2	2.8	0.54	0.37	0.53
Tyrosine, μmol/L							
24 h postpartum	108.0	105.6	122.8	8.5	0.18	0.13	0.28
d 21 of lactation	111.2^{ab}	84.8^{b}	119.6^{a}	7.1	0.06	0.52	0.02
Valine, μmol/L							
24 h postpartum	258.2	267.8	291.0	19.0	0.54	0.31	0.72
d 21 of lactation	245.0^{ab}	190.6^{b}	292.6^{a}	18.3	0.06	0.23	0.04
Trp/LNAA[3] ratio							
24 h postpartum	0.0867	0.0892	0.0969	0.004	0.86	0.60	0.96
d 21 of lactation	0.0751^{b}	0.0919^{a}	0.0716^{b}	0.003	0.02	0.58	<0.01

[1] Blood samples from 4 sows per treatment.
[2] Standard error of mean.
[3] Large neutral amino acid (sum of Ile, Leu, Val, Phe, and Tyr).
^{abc} means with different superscripts within the same row significantly differ (P<0.05).

Table 12. Effects of dietary tryptophan levels in gestation on milk composition and estimated nutrient output of sow milk

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Milk composition							
Casein, %							
24 h postpartum	5.88	6.77	7.71	0.698	0.74	0.34	0.84
d 21 of lactation	4.49	4.49	4.52	0.041	0.95	0.82	0.83
Fat, %							
24 h postpartum	5.83	8.50	7.96	0.799	0.29	0.24	0.17
d 21 of lactation	7.20	7.22	6.72	0.287	0.77	0.55	0.71
Protein, %							
24 h postpartum	7.59	8.66	9.93	0.945	0.76	0.36	0.82
d 21 of lactation	4.87	4.89	4.91	0.045	0.97	0.81	1.00
Lactose, %							
24 h postpartum	4.51	4.35	4.41	0.138	0.88	0.66	0.94
d 21 of lactation	6.25	6.20	6.32	0.052	0.75	0.69	0.53
Total solid, %							
24 h postpartum	20.26	24.30	25.28	1.498	0.47	0.20	0.71
d 21 of lactation	19.55	19.62	19.13	0.298	0.81	0.61	0.70
Solid not fat, %							
24 h postpartum	12.46	13.26	14.66	0.812	0.73	0.33	0.73
d 21 of lactation	11.11	11.17	11.22	0.044	0.67	0.39	0.94
Free fatty acid, %							
24 h postpartum	3.87	4.73	4.14	0.246	0.30	0.62	0.08
d 21 of lactation	6.16	5.40	6.88	0.459	0.50	0.57	0.32
Estimated nutrient output of sow milk[3], piglet/day							
Milk production[4], g	702.6	733.5	727.6	12.74	0.52	0.28	0.72
Milk dry matter[5], g	130.6	135.5	134.8	2.05	0.52	0.28	0.75
Milk energy[6], kcal	856.0	887.0	881.0	12.92	0.53	0.29	0.72

[1] Milk samples from 4 sows per treatment.
[2] Standard error of mean.
[3] Noblet and Etienne, 1989.
[4] Milk production (g/piglet/day)=2.50*ADG (piglet average daily gain, g) + 80.2*BW_i (piglet BW at the beginning, kg) + 7.
[5] Milk dry matter (g/piglet/day)=0.40*ADG + 12.9*BW_i + 19.
[6] Milk energy (kcal/piglet/day)=2.54*ADG + 78.7*BW_i + 153.

Table 13. Effects of dietary tryptophan levels in gestation on protein, fat, and energy contents and their changes in sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Protein[3], kg							
Gestation							
d 0	33.79	33.75	33.54	0.360	0.95	0.76	0.90
d 110	38.07	36.49	36.46	0.617	0.66	0.56	0.46
Changes (d 0 - 110)	4.38	3.21	2.99	0.486	0.64	0.42	0.58
Lactation							
24 h postpartum	35.37	34.23	33.72	0.502	0.34	0.15	0.99
d 21 of lactation	34.07	33.00	32.64	0.499	0.41	0.19	0.92
Changes (d 0 - 21)	-1.30	-1.23	-1.08	0.197	0.86	0.63	0.83
Fat[4], kg							
Gestation							
d 0	44.87	44.01	43.77	1.314	0.94	0.74	0.91
d 110	57.54	51.99	54.23	1.791	0.37	0.34	0.27
Changes (d 0 - 110)	12.15	9.84	10.56	1.186	0.47	0.26	0.59
Lactation							
24 h postpartum	50.09	46.87	50.51	1.801	0.73	0.79	0.47
d 21 of lactation	46.03	44.01	46.55	1.632	0.83	0.79	0.59
Changes (d 0 - 21)	-4.06	-2.86	-3.96	0.858	0.90	0.96	0.65
Energy[5], MJ							
Gestation							
d 0	2659.64	2623.37	2608.93	57.189	0.93	0.72	0.93
d 110	3289.18	3021.22	3112.14	82.523	0.35	0.32	0.25
Changes (d 0 - 110)	610.74	485.38	509.80	52.609	0.42	0.23	0.54
Lactation							
24 h postpartum	2914.43	2752.65	2888.81	81.178	0.80	0.99	0.52
d 21 of lactation	2715.15	2603.98	2699.16	74.167	0.88	0.98	0.62
Changes (d 0 - 21)	-199.28	-148.67	-189.65	38.266	0.93	0.98	0.70

[1] Dourmad et al., 2008.
[2] Standard error of mean.
[3] Protein content of sow = 2.28 + 0.178*empty body weight (0.96*BW, kg) − 0.333*BF thickness (at P_2 site, mm).
[4] Fat content of sow = −26.4 + 0.221* empty body weight + 1.331*BF thickness.
[5] Energy content of sow = −1074 + 13.65* empty body weight + 45.94*BF thickness.

Chapter Ⅳ. Effects of Dietary Tryptophan Levels in Lactating Sows on Performance and Blood Metabolites

Abstract

This experiment was conducted to evaluate the effects of dietary Trp levels in lactating sows on the reproductivity and blood metabolites. A total of 30 mixed-parity (average 4.73) lactating sows (Yorkshire×Landrace) with an initial BW of 234.27±17.35kg were used in a 3 week trial. Sows were allotted to one of three treatments in a completely randomized design by their BW, BF thickness, parity and litter weight. Each treatment was designed based on relative ratio Trp to Lys in the lactation diet. Treatments were as followed: 1) Trp18%: 18% *ai* Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. Experimental diets contained 2,350 kcal NE/kg, 13.5% CP, 0.65% *ai* Lys, 62% *ai* TSAA/*ai* Lys, and 75% *ai* Thr/*ai* Lys, as fed basis. All other nutrients in experimental diet were met or exceeded the NRC requirement (2012). Although Trp22% and Trp26% treatments showed higher daily feed intake in the middle of lactation (8 ~ 14 d) than Trp18% treatment, there were no differences in BW, BF thickness, ADFI, and WEI among treatments during the whole lactation. At farrowing, dietary Trp levels did not affect number of piglets in total born, still born, mummy and born alive. After cross-fostering, Trp22% and Trp26% treatments showed the higher litter weight at d 21 of lactation compared with Trp18% treatment (P=0.08). In addition, the linear increases were observed in litter weight at d 21 of lactation and litter weight gain as dietary Trp level was increased ($P=0.06$ and $P=0.04$, respectively). But, piglet BW and BW gain during lactation were not affected by dietary Trp level. In blood metabolites of sows, there were no differences in the concentration of BUN, cortisol, and melatonin among treatments. Higher serotonin concentration was observed in Trp22% and Trp26% treatments than Trp18% treatment at 24 h postpartum (P=0.03). As dietary Trp level increased, serotonin concentration at 24 h postpartum increased (linear, P=0.02). At 24 h postpartum, blood cortisol tended to decrease whereas blood melatonin was numerically increased as dietary Trp level of lactation diet was increased (P=0.56 and

P=0.16, respectively). In addition, BUN, cortisol, and glucose level in blood of nursing piglets were not affected by dietary Trp level in lactation sow's diet. Trp concentration and Trp/LNAA level of blood had no difference among treatments. No differences were observed in casein, fat, protein, lactose, total solid and solid not fat content of sow milk among treatments. Furthermore, milk production, milk DM, and milk energy were not affected by dietary Trp level in lactation. There were no significant differences in protein, fat, and energy contents of sows and their changes during lactation. As a consequence, high dietary Trp level in lactation feed (above 22% *ai* Trp/*ai* Lys, 0.65% *ai* Lys) increased the serotonin level of blood at farrowing and improved litter weight gain with elevating feed intake in the middle of lactation. Nevertheless any improvements were not found in BW and BF thickness change of sows, piglet BW gain, ADFI, and WEI when sows were fed diets containing *ai* Trp/*ai* Lys above 22% during the whole lactation. These results indicated that maximal ratio of *ai* Trp/*ai* Lys in lactation diet was 18% (0.65% *ai* Lys in lactation feed).

Key words: Tryptophan, Lactating sow, Reproductive performance, Tryptophan ratio per lysine, Blood metabolites

Introduction

Lactating sow is on the catabolic condition due to the negative nutrient balance during lactation (Kim et al. 2013). Catabolic status in sow increases the production of reactive oxygen species causing increased oxidative stress (Bernardi et al, 2008, Berchieri-Ronchi et al., 2011). High oxidative stress in sows caused the impaired milk production, reproduction performance and longevity of sows (Flowers and Day, 1990; Zhao, 2011a; Zhao, 2011b). However, dietary tryptophan had a positive effect on immune response for health maintenance (Moffet and Namboodiri, 2003; Le Floch and Seve, 2007). So, effect of dietary tryptophan was anticipated to reduce the oxidative stress and lactating stress.

Reducing catabolism of lactating sows originated from milk production, improving daily feed intake is needed for lactating sow (Noblet et al., 1990). Insufficient feed intake during lactating is caused to greater body weight loss, decreased milk production and reproductive problems (Baidoo et al., 1992; Eissen et al., 2000). Trp is metabolized to melatonin, which might serve as a signal for regulation of appetite or synchronization of the feeding and digestion processes (Bubenik et al., 1996) and increasing feed intake (Inui et al., 2004; Sakata and Sakai, 2010; Dickson et al., 2011).

Poletto et al. (2010) reported that higher level of dietary tryptophan reduced the behavioral activity and aggressive action in gilts. In addition, Muns et al. (2010) indicated that high dietary tryptophan level showed lower number of behavioral position change at 6-7 postpartum. High dietary tryptophan level is expected to relieve status of sows and reduce incidence of crushed piglet.

Therefore, this study was conducted to evaluate the effect of dietary tryptophan level on physiological changes, reproductive performance, litter performance, blood profiles and milk production in lactating sows.

Materials and Methods

Animal and housing

A total of 30 gestating sows (Yorkshire×Landrace, average parity 4.73) with an

initial BW of 234.27±17.35 kg was used in a 3 week lactation experiment. Sows were allotted to 1 of 3 treatments in a completely randomized design by their BW, BF thickness, parity and litter weight. When d 110 of gestation, sows were moved into individual farrowing crates (2.5m×1.8m) and housed until weaning. Before 5 days of farrowing, each experimental diet was provided to sows for adaptation with reducing the diet 200 g/d from 2.4 kg/d (over 3rd parity) and 2.2 kg/d (2nd parity) until predicted day of parturition. After parturition, feed intake increased gradually by 1 kg/d until 5 day postpartum (5 kg at 5 day postpartum). After 5 day postpartum, feed and water were provided *ad libitum* to sows. Within 24 h postpartum, Fe-dextran (150 ppm) injection, ear notching, needle teeth clipping and tail docking were practiced to all piglets. Piglets were cross-fostered across treatments within 1 day after birth to balance suckling intensity of sows with equalization of litter size, and thus to minimize impact of initial litter size potentially affecting litter growth. In addition, male piglets were castrated in d 3 postpartum.

Treatment and experimental diet

Each treatment was designed based on relative ratio Trp to Lys in the lactation diet. Treatments were as followed: 1) Trp18%: 18% *ai* (apparent ileal digestible) Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. Experimental diets contained 2,350 kcal NE/kg, 13.5% crude protein, 0.650% *ai* Lys, 62% *ai* TSAA/*ai* Lys, and 75% *ai* Thr/*ai* Lys. Also calcium and total phosphorus content was 0.90 and 0.60, respectively. All nutrients were met or exceeded NRC (2012) nutrient requirement. The formula and chemical composition of experimental diets were presented in Table 14.

Measurements

Body weight and BF thickness of sows were measured at 24 h postpartum and d 21 of lactation. Body weight of sows was measured with weighing machine (CAS, Korea). An ultra-sound device (Renco lean meter, Renco Corp., Minneapolis, USA) was used for measuring BF thickness at P_2 position (mean value from both side of the last rib and 65 mm away from the backbone). The numbers of total born, stillborn, mummy and born alive were recorded within 24 h postpartum and the number of piglets per litter was recorded after cross-fostering and at d 21 of lactation. Piglet weight was recorded within 24 h postpartum

and at d 21 of lactation. Litter weight was calculated by summing the individual piglet weights from one sow. Feed intake was recorded during experimental period (0-3 week) and feed waste was checked frequently when sows fed the experimental diet. WEI was determined by monitoring for first estrus after weaning.

Blood samples were collected from the anterior vena cava of 5 sows and 5 piglets from each treatment at 24 h postpartum and d 21 postpartum. All samples were enclosed into collecting tubes (serum and EDTA tube, BD Vacutainer®) and centrifuged at 3,000 rpm and 4℃ for 5 minutes after clotting at room temperature for 30 minutes (serum) or mixing sufficiently (plasma). The upper liquid (serum) of the blood was separated to a microtube (Axygen, Union City, CA, USA) and stored at -20℃ until later analysis. Cortisol and melatonin were analyzed by ELISA kit (Endocrine technologies, USA; IBL, Germany) and serotonin was analyzed by HPLC (Recipe, Germany). BUN and glucose were analyzed by kinetic assay (Modular analytics, Roche, Germany) and FFA and total protein were analyzed by colorimetry method (Moldular analytics, Hitachi, Japan). Amino acid was analyzed by LC-MS/MS method (LC-MS/MS, 3200 Q TRAP, AB SCIEX, USA).

Colostrum and milk samples were taken from the 5 sows of each treatment at 24 h postpartum and d 21 of lactation, respectively. After injection of oxytocin (Komioxytocin inj., Komipharm international Co. Ltd., Siheung, Korea) 0.3ml (10IU/ml), milks were collected from first or second teat of sow. After milk collection in conical tube (50ml), samples were stored in a freezer (-20℃) until further analysis. Proximate analysis of colostrum and milk was conducted using Milkoscan FT 120 (FOSS Electric, Sungnam, Korea). The milk production (g), milk dry matter (g) and milk energy were calculated from piglet weights and growth rates by equations in Noblet and Etienne (1989). The prediction of fat (kg), energy (MJ) and protein (kg) content of sows (lactation d 0 and 21) was from empty body weight (EBW=0.96*BW, kg) and backfat thickness (P_2 site, mm) by the equation of Dourmad et al. (2008).

Statistical analysis

All statistical analyses by least squares mean comparisons were carried out using the PDIFF option with the General Linear Model procedure of SAS (SAS Institute, 2004).

Orthogonal polynomial contrasts were used to determine linear and quadratic effects by increasing Trp levels in lactation for all measurements of sows and piglets, blood profile and milk composition. Individual sows were used as the experimental unit in performance and each sample was used as the experimental unit in blood and milk analysis. Probability values less than 0.05 ($P<0.05$) were considered as significant difference; $0.05 \leq P < 0.10$ were indicative of a trend; and values equal to or greater than 0.10 were considered as non-significant difference.

Results and Discussion

Reproductive and litter performance

Effect of dietary Trp levels on reproductivity and litter performance in lactating sows were presented in Table 15. At farrowing, dietary Trp levels did not affect on number of piglets in total born, still born, mummy and born alive. After cross-fostering, Trp22% and Trp26% treatments showed the higher litter weight (70.12 kg and 69.70 kg, respectively) at d 21 of lactation compared with Trp18% treatment (63.76 kg, $P=0.08$). In addition, the linear increases were observed in litter weight at d 21 of lactation and litter weight gain as dietary Trp level was increased (linear, *P=0.06* and *P=0.04*, respectively). But piglet weight and weight gain during lactation were not affected by dietary Trp level.

Previous studies have tried to determine the effects of dietary Trp level on lactating sows. In the current study, litter weight at d 21 of lactation and weight gain was increased linearly as Trp supplementation level increased, which was in disagreement with results of Libal et al. (1997). Previous study showed that high dietary Trp level (22.6% Trp/Lys, 0.75% Lys) treatment showed similar results compared with control treatment (16.0% Trp/Lys, 0.75% Lys) in litter weight and litter weight gain. In piglet weight gain, the present study showed no difference among treatments. Lewis and Speer (1974) studied the effect of different Trp level on lactating sow. Dietary Trp level (> 0.066% in lactation feed) showed plateau effect in piglet weight gain (d 7 ~ 21 of lactation) until 0.0161% dietary Trp level (2.10 kg and 2.38 kg, respectively). However, 0.046% dietary Trp level decreased piglet weight gain up to 32% (0.046% vs. 0.066%, 1.59 kg vs. 2.10 kg). As total basis, dietary Trp level of the present

study ranged from 0.155% (Trp18% treatment) to 0.208% (Trp26% treatment). Trp levels of current study exceeded an inflection point of the previous finding, which may cause no effect in piglet weight gain. Number of weaned piglets of Trp22% and Trp26% treatments were higher than Trp18% treatment and high Trp level had lower piglet death (no. of pigs) during lactation as followed treatments; Trp26%, Trp22%, and Trp18%: 0.9 pigs (0.4, 0.6, and 0.9, respectively). Various numbers of piglets at d 21 may cause differences in litter weight and litter weight gain among treatments. In summary, high Trp level (above 18% *ai* Trp/*ai* Lys , 0.65% *ai* Lys) could not be beneficial on growth performance of nursing piglet and additional studies are needed to demonstrate the effect of Trp on mortality of nursing piglets.

Daily feed intake of Trp22% and Trp26% treatments were higher than Trp18% treatment on the middle of lactation (d 8 - 14) numerically (Figure 10), there were no differences in ADFI during whole lactation and WEI among treatments. Libal et al. (2007) demonstrated that feed intake was greater for sows fed 0.17% dietary Trp (22.6% Trp/Lys) than for sows fed 0.12% Trp (16.0% Trp/Lys) at the 3rd week and overall period. Stahly et al. (1990) and Tokach et al. (1991b) have reported increases in sow reproductivity due to feeding higher crude protein diet during lactation. Roth-Maier et al. (2004) studied about Trp level (SID (standardized ileal digestible) Trp, 0.09%, 0.12%, 0.15%, 0.21%, 0.27%, 0.39%; SID Lys level, 0.88%) in lactating sows. They showed that increasing SID Trp concentrations in the diet from 0.9 to 2.1 g/kg feed increases feed intake up to 2 times (10.0% vs. 23.8% SID Trp/Lys, respectively).

In contrast, Muns R. et al, (2010) reported total feed intake (116.36 vs. 118.43 kg, 21days after farrowing) was not affected by dietary Trp level between treatments (0.20 vs. 0.26%, total Trp level in lactation diet), which is in agree with the present study. In parallel as well as other phase of pig, Li et al., (2006) reported that feed intake of 2 or 4 times higher Trp supplemented treatments were not different compared with control treatment.

Poor body condition after lactation had negative impact on WEI (Vesseur et al., 1994) and eventually negative impact on reproductivity of following parity. Low feed intake due to marginal or limiting Trp levels have been reported in growing pig by Arentson and Zimmerman (1985), Lin et al. (1987), and Uttecht et al. (1991). Furthermore, the adverse effect of feeding a Trp-deficient diet on feed intake in sows was well documented (Lewis and Speer, 1974; Easter and Baker, 1977). Several researchers have demonstrated that sows

consuming low amounts of feed during lactation have a delayed return to estrus after weaning. For example, Pettigrew and King (1992) conducted an experiment during which sows were fed various amounts of feed during 28 days of lactation. The percentage of sows displaying estrus within 8 days after weaning was 8%, 33%, 50%, 58%, 58%, and 83%, for sows consuming 1.5 kg, 2.2 kg, 2.9 kg, 3.6 kg, 4.2 kg and 4.8 kg of ADFI during lactation, respectively. The interval from weaning to the onset of estrus was negatively correlated with daily feed intake during lactation (Estienne et al., 2003). In this study, ADFI was not different among treatments and ranged from 6.65 ~ 6.71 kg. This result suggests that ADFI and WEI were not affected by dietary Trp level when sow diet contained Trp above 18% *ai* Trp/*ai* Lys (0.65% *ai* Lys in lactation diet).

Body weight and backfat thickness

Effect of dietary Trp levels on body weight and backfat thickness in lactating sows were presented in Table 16. There were no significant differences in BW and BF thickness at 24 h postpartum and d 21of lactation among treatments. In addition, changes of BW and BF thickness were not affected by dietary Trp level. Lewis and Speer (1974) reported that dietary Trp levels during lactation did not affect BW changes and BF thickness changes. On the other hand, Libal et al. (1997) demonstrated that sows fed low Trp level showed a greater weight loss than those fed high Trp level (0.12% vs. 0.18%, respectively). The calculated dietary Trp ratio per Lys in the previous study was 22.6% (0.18% dietary Trp level) and 16.0% (0.12% dietary Trp level). Dietary Trp/Lys level over 18% did not affect BW loss and BF thickness loss significantly during lactation. In accordance with result of Muns R. et al, (2010), no significant differences between treatments were observed for BCS, BF thickness and feed intake in sows.

Increased feed intake due to increasing dietary protein has been reported for lactating sows (Mahan and Grifo, 1975; Mahan and Mangan, 1975; NCR-42 Committee ,1978). In contrast, Johnston (1991) reported increasing levels of protein from 13.6 to 19.2% did not affect feed intake of high-producing sows, but a correlation between increasing dietary protein and reduction of sow weight loss was observed. In summary, high Trp level in lactation diet (> 18% *ai* Trp/*ai* Lys, 0.65% *ai* Lys) did not affect on body weight and backfat thickness of sows because of similar ADFI among treatments.

Blood metabolites

Effect of Trp supplementation level on blood metabolites in lactating sows and nursing piglets were shown in Table 17. There were no differences in the concentration of blood urea nitrogen (BUN), cortisol, and melatonin. In serotonin concentration, Trp22% and Trp26% treatments showed higher levels than Trp18% treatment at 24 h postpartum (P=0.03). As dietary Trp level increased, serotonin concentration at 24 h postpartum increased (linear, P=0.02). At 24 h postpartum, there were reduction of cortisol and elevation of melatonin in blood numerically (P=0.56 and P=0.16, respectively) as dietary Trp level in lactation was increased. In addition, BUN, cortisol, and glucose level in blood of nursing piglets were not affected by dietary Trp level.

Little Studies have tried to determine the effects of dietary Trp level on blood metabolites of lactating sows. Poletto et al. (2010) reported the effect of additional dietary Trp on the behavior and aggressive in grower gilt. They found that higher level of supplement of Trp in the diet reduced the behavioral activity and the aggressive action of grower gilts with increasing plasma serotonin concentration (up to 12.8 ~ 20.3%). Roth-Maier et al (2004) conducted about the effects of various Trp levels (SID (standardized ileal digestible) Trp, 0.09%, 0.12%, 0.15%, 0.21%, 0.27%, 0.39%; SID Lys level, 0.88%) on lactating sows. They showed that increasing SID Trp concentrations in the diet from 0.9 to 3.9 g/kg feed increased serum serotonin during lactation. Cortisol was measured in the present experiment because of the pain due to uterine contractions and piglet birth may be stressful for sows (Lawrence et al., 1994; Jarvis et al., 1998). Mosnier et al. (2009) demonstrated that plasma cortisol concentration was increased 2 times 10 days around farrowing. Kim et al. (2015) suggested that short-term supplementation of L-Trp (0.8% Trp in diet) associated with reduced salivary cortisol concentrations in grower pig at 6 day after feeding of 0.8% Trp (2.79 vs. 2.06, ng/ml, P=0.05). Serotonin in turns directly blocks cortisol in rainbow trout (Lepage et al., 2002). Trp was not only metabolized to serotonin, but subsequent metabolism of serotonin resuled in the formation of melatonin. These results suggested that high dietary Trp (above 0.18% dietary Trp level (as > 0.14% as *ai* Trp) in lactation diet) induced more secretion of melatonin and serotonin and it may mitigate the stress from parturition. However, more studies are needed to demonstrate the effect of dietary Trp level on farrowing stress of lactating sow.

Amino acid profiles in blood

Effect of dietary Trp levels on amino acid profiles of sows' blood was presented in Table 18. Trp concentration and Trp/LNAA level of blood were not different among treatments. Little studies were conducted to demonstrate the effect of dietary Trp level on blood Trp and LNAA concentration in lactating sows. Libal et al. (1997) showed that the dietary Trp level (0.12% vs. 0.17%, 0.75% Lys in lactation feed) had affected the plasma Trp, Phe, and Val concentrations (0.26 vs. 1.18, 0.75 vs. 1.25, and 1.60 vs. 2.23, mg/dl, respectively).

Plasma Trp concentrations reflected dietary Trp supplementation in several previous studies (Meisinger and Speer, 1979; Arentson and Zimmerman, 1985; Uttecht et al., 1992; Libal et al., 1997). In the present study, plasma Trp concentration and Trp/LNAA ratio were not different among treatments, which is in disagreement with previous findings. When disease was challenged, high immune status caused an increased demand of Trp then for the synthesis of proteins for an immune response (Le Floc'h, 2009). Dapoza (2009) also reported that Trp level in the diet can be increased, especially in poor health condition. The decreases in plasma Trp concentration (higher Trp requirement) in pigs housed in poor sanitary conditions reflect the specific use of this amino acid to satisfy the immune functions of the animal (Melchior et al., 2004). These previous studies suggest stresses increase demand of Trp, furthermore reduce plasma Trp concentration, which is in disagreement with present result. The current study showed the high level of serotonin in plasma at farrowing with no differences of Trp concentration among treatments as dietary Trp level increased.

Supplementation of Trp in lactating sows' diet had no significant effects on Trp/LNAA ratio at d 21of lactation (Table 18). Markus (2007) suggested that cold-presser stress significantly increased cortisol, reduced mood, and cognitive performance, whereas carbohydrates significantly increased plasma Trp/LNAA (30%) and positively affected performance and mood under stress. This result suggested that carbohydrate level of diet might affect plasma Trp/LNAA, which is insufficient for the present study because of no difference in ADFI among treatments. Lewis and Speer (1974) studied that total concentration LNAA (Ile, Leu, Phe, and Val) of plasma (9.71, 8.27, 7.46, 7.37 and 7.98, mg/100ml, respectively) showed plateau affected by dietary Trp level increased (0.046, 0.066, 0.091, 0.121 and 0.161, %, dietary Trp). Further studies would be needed to

demonstrate the relationship between dietary Trp and Trp concentration, Trp/LNAA ratio, and stress indicators of plasma in sows.

Milk composition and estimated nutrient output

Effects of Trp supplementation level on milk composition and estimated nutrient output were presented in Table 19. No differences were observed in casein, fat, protein, lactose, total solid and solid not fat content of sow milk among treatments. Furthermore, milk production, milk dry matter (DM), and milk energy were not affected by dietary Trp level in lactation. Lewis and Speer (1974) showed that dietary Trp levels (0.046, 0.066, 0.091, 0.121 and 0.161, %, dietary Trp level) did not affect total solids concentration of milk on 15 d and d 21 of lactation (15.36, 16.06, 15.94. 15.77 and 15.98, %, mean value). After low energy supply during gestation, body reserve mobilization for milk production in sows cannot be compensated by higher energy supply during lactation (Beyer et al. 2007), which is in accordance with result of current study. This study showed that the BW, BF thickness and ADFI were not different during lactation as dietary Trp levels in lactation. In summary, *ai* Trp/*ai* Lys above 18% (0.65% *ai* Lys) in lactation did not affect the body reserve mobilization for milk production during lactation.

In addition, Lewis and Speer (1974) indicated that dietary Trp level improve milk yield during the first 3 weeks of lactation and the effect was particularly evident at the lowest level of Trp under the restricted 5.45kg daily feed intake. This result had no effect on milk production, milk DM, and milk energy content. Consequently, above 18% *ai* Trp/*ai* Lys (0.65% *ai* Lys) in lactation feed did not affect the body reserve mobilization for milk production during lactation.

Protein, fat, and energy contents and their deposition

Effect of Trp supplementation on protein, fat, and energy deposition in lactating sows were presented in Table 20. There were no significant differences in protein, fat, and energy contents of sows and their changes during lactation. In the result of Lewis and Speer (1974), increasing dietary Trp resulted in significant linear increases in daily nitrogen retention. When sows were fed various Trp diets (0.46, 0.66, 0.91, 1.21 and 1.61, Trp g/kg) during lactation, they showed the plateau trend (>0.91 g/kg) of nitrogen retention (16.4, 27.2,

36.2, 35.5 and 36.1, g, respectively). Trp levels of this study (1.55 ~ 2.08 total Trp g/kg) exceeded the highest level of the previous finding, which may cause no effect on the protein deposition. Moehn et al. (2011) suggested that restricted and phase feeding during gestation can influence body condition of sow. Energy intake is the limiting factor on fat deposition and fat mobilization. Presumably energy intake among treatments was similar in current study, because ADFI during lactation was not different among treatments with same energy content of each treatment. Consequently, *ai* Trp/*ai* Lys above 18% (0.65% *ai* Lys) on lactation diet had no effect on deposition of protein, fat, and energy due to no differences of feed intake among treatments.

Conclusion

When sows were fed lactation diet over 18% *ai* Trp/*ai* Lys, ADFI was increased on the middle of lactation (d 8 - 14) numerically. However there were no differences in BW, BF thickness, ADFI, and WEI among treatments during whole lactation. At farrowing, dietary Trp levels did not affect on the number of piglets in total born, still born, mummy and born alive. After cross-fostering, the linear increases were observed in litter weight at d 21 of lactation and litter weight gain as dietary Trp levels were increased. But, piglet weight and weight gain during lactation were not affected by dietary Trp level. BUN, cortisol, and melatonin of sows' blood showed no differences among treatments. BUN, cortisol, and glucose level of blood in nursing piglets were not affected when sows were fed lactation diet above 18% *ai* Trp/*ai* Lys. No differences were observed in milk composition, milk production, milk DM, and milk energy among treatments. Also, there were no significant differences in protein, fat, and energy contents of sows and their changes during lactation.

In summary, the present study showed that high dietary Trp level (above 22% *ai* Trp/*ai* Lys, 0.65% *ai* Lys) in lactation feed increased the serotonin level of blood at farrowing and improved litter weight gain with elevating feed intake on the middle of lactation. Nevertheless, BW and BF change of sows, piglet weight gain, ADFI, and WEI were not improved when sows were fed diets containing *ai* Trp/*ai* Lys above 22% during whole lactation. These results can lead to the conclusion that maximal level of *ai* Trp/*ai* Lys

in lactation diet was 18%. Further studies would be needed to demonstrate the effects of less dietary Trp level than 18% *ai* Trp/*ai* Lys for lactating sows and plasma AAs concentration.

Table 14. Formula and chemical composition of experimental diet in lactation

Items	Treatment[1]		
	Trp18%	Trp22%	Trp26%
Ingredient, % (as fed basis)			
Corn	40.20	39.76	39.34
Wheat	30.00	30.00	30.00
Soybean meal-45%CP	10.62	10.64	10.66
Rapeseed meal	3.00	3.00	3.00
Wheat bran	3.50	3.50	3.50
Soy hull	3.62	3.64	3.66
Mixed animal fat	2.70	2.82	2.94
Molasses	2.00	2.00	2.00
L-lysine HCl	0.73	0.73	0.73
DL-Methionine 99%	0.03	0.04	0.04
L-tryptophan 10%[2]	0.07	0.34	0.60
L-threonine 99%	0.09	0.09	0.09
Mono-di calcium phosphate	1.14	1.14	1.14
Limestone	1.29	1.29	1.29
Salt	0.50	0.50	0.50
Vitamin and mineral premix[3]	0.46	0.46	0.46
Phytase (1,000 IU)	0.05	0.05	0.05
Chemical composition (calculated values, as fed basis)			
NE, Kcal/kg	2,350	2,350	2,350
Crude protein, %	13.5	13.5	13.5
Total Lys, %	0.780	0.780	0.780
Total Trp, %	0.155	0.182	0.208
ai Lys, %	0.650	0.650	0.650
ai Trp, %	0.117	0.143	0.169
ai Met + Cys / *ai* Lys, %	62	64	64
ai Thr / *ai* Lys, %	65	65	65
ai Trp / *ai* Lys, %	18	22	26
Ca, %	0.9	0.9	0.9
Total P, %	0.6	0.6	0.6

[1] Trp18% (*ai* Trp/*ai* Lys, 18%), Trp22% (*ai* Trp/*ai* Lys, 22%), and Trp26% (*ai* Trp/*ai* Lys, 26%).
[2] Feed grade (CJ, Indonesia).
[3] Supplied the following per kilogram of diet: vitamin A, 18,030 IU; vitamin D_3, 3,600 IU; choline chloride, 1,200 mg; vitamin E, 100 IU; cyanocobalamine, 40 mg; niacin, 20.0 mg; Ca panthotenate, 16.0 mg; riboflavin, 4.0 mg; menadione, 4.0 mg; pyridoxine, 2 mg; folic acid, 1 mg; thiamine 0.8 mg; d-biotin, 0.2 mg; Fe, 200.0 mg (as $FeSO_4$); Mn, 79.0 mg (as $MnSO_4$); Zn, 35.0 mg (as $ZnSO_4$); Cu, 5.0 mg (as $CuSO_4 5H_2O$); Co, 1.98 mg (as $CoSO_4$); I, 1.26 mg (as $Ca(IO_3)$); Se, 0.1 mg (as Na_2SeO_3).

Table 15. Dietary tryptophan levels in lactation on farrowing, their progeny growth performance, average daily feed intake and weaning to estrus interval in sows

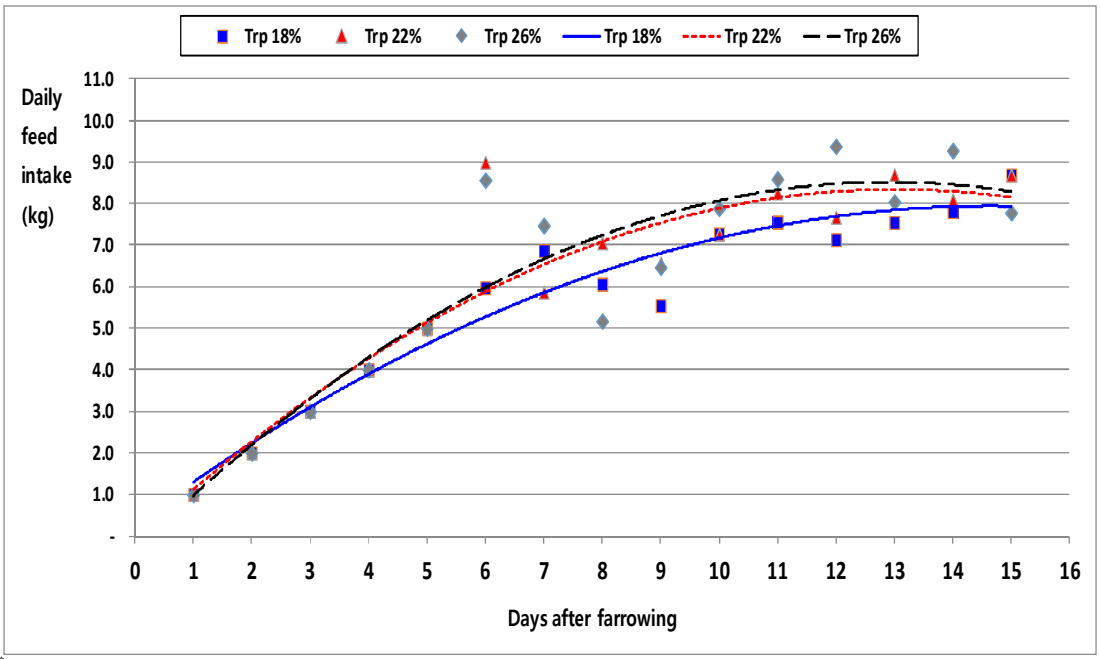
Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
No. of piglets							
Total born	11.7	13.2	11.9	0.70	0.70	0.92	0.41
Stillborn	0.4	0.5	0.5	0.17	0.97	0.83	0.90
Mummy	0.2	0.6	0.3	0.16	0.59	0.81	0.33
Born alive	11.1	12.1	11.1	0.56	0.77	1.00	0.47
After cross-fostering	---------- 11.2 ----------						
d 21 of lactation	10.2	11.0	10.6	0.19	0.30	0.43	0.18
Litter weight, kg							
24 h postpartum	17.35	18.40	17.53	0.475	0.60	0.87	0.33
d 21 of lactation	63.76	70.12	69.70	1.507	0.08	0.06	0.20
Weight gain (d 0 - 21)	46.41	51.72	52.17	1.397	0.09	0.04	0.31
Piglet weight, kg							
24 h postpartum	1.52	1.62	1.56	0.036	0.61	0.69	0.37
d 21 of lactation	6.23	6.33	6.49	0.094	0.54	0.28	0.89
Weight gain (d 0 - 21)	4.71	4.71	4.93	0.098	0.61	0.38	0.65
Average daily feed intake, kg/d							
	6.65	6.66	6.71	0.103	0.97	0.83	0.94
Weaning to estrus interval, d							
	3.87	4.43	4.06	0.16	0.32	0.64	0.16

[1] A total of 30 mixed-parity lactating sows (Yorkshire×Landrace, average parity 4.73) with an initial BW of 234.27±17.35 kg.
[2] Standard error of mean.

Table 16. Effect of dietary tryptophan levels in lactation on body weight and backfat thickness in lactating sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Body weight, kg							
24 h postpartum	234.1	233.1	236.7	3.17	0.90	0.76	0.75
d 21 of lactation	231.6	227.7	231.6	3.37	0.87	1.00	0.60
BW changes (d 0 - 21)	-3.5	-5.4	-5.1	1.49	0.67	0.49	0.58
Backfat thickness, mm							
24 h postpartum	21.3	20.9	21.0	0.84	0.97	0.87	0.87
d 21 of lactation	20.7	19.5	20.6	0.77	0.69	0.95	0.40
BF changes (d 0 - 21)	-0.6	-1.4	-0.4	0.42	0.56	1.00	0.29

[1] A total of 30 mixed-parity lactating sows (Yorkshire×Landrace, average parity 4.73) with an initial BW of 234.27±17.35 kg.
[2] Standard error of mean.



* A total of 30 mixed-parity lactating sows (Yorkshire×Landrace, average parity 4.73) with an initial BW of 234.27±17.35 kg

Figure 10. Effect of dietary tryptophan levels in lactation on daily feed intake during early lactation

Table 17. Effect of dietary tryptophan levels in lactation on blood metabolites of sows and their progeny in lactation

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Sows							
Blood urea nitrogen, mg/dL							
24 h postpartum	12.30	12.66	10.76	1.020	0.74	0.56	0.62
d 21 of lactation	16.42	15.00	14.86	0.754	0.75	0.47	0.61
Cortisol, ng/mL							
24 h postpartum	6.46	5.97	4.18	0.955	0.74	0.56	0.69
d 21 of lactation	5.38	7.57	6.26	0.898	0.70	0.70	0.33
Melatonin, pg/mL							
24 h postpartum	56.02	69.31	78.39	5.77	0.34	0.16	0.87
d 21 of lactation	71.35	62.90	68.88	5.76	0.76	0.73	0.53
Serotonin, ng/mL							
24 h postpartum	151.2[b]	213.8[a]	229.2[a]	15.66	0.03	0.02	0.32
d 21 of lactation	280.5	289.8	322.3	32.31	0.87	0.56	0.89
Nursing piglets							
Blood urea nitrogen, mg/dL							
24 h postpartum	22.64	20.90	19.82	1.361	0.78	0.50	0.93
d 21 of lactation	6.50	6.28	5.86	0.347	0.88	0.67	0.84
Free fatty acid, uEq/L							
24 h postpartum	282.8	222.6	307.2	24.50	0.40	0.90	0.20
d 21 of lactation	483.8	496.0	394.2	24.27	0.28	0.20	0.33
Glucose, mg/mL							
24 h postpartum	108.3	109.2	114.4	6.457	0.97	0.89	0.84
d 21 of lactation	127.8	133.8	130.8	2.291	0.59	0.61	0.38

[1] Blood samples from 4 sows per treatment.
[2] Standard error of mean.
[abc] means with different superscripts within the same row significantly differ ($P<0.05$).

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Table 18. Effect of dietary tryptophan levels in lactation on amino acid profiles in blood plasma of lactating sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Arginine, μmol/L							
24 h postpartum	196.4	231.2	211.0	9.74	0.32	0.52	0.18
d 21 of lactation	135.4	150.3	188.4	11.13	0.24	0.11	0.66
Histidine, μmol/L							
24 h postpartum	117.4	116.4	114.8	3.36	0.93	0.72	0.96
d 21 of lactation	104.4	103.8	109.2	3.60	0.78	0.65	0.61
Isoleucine, μmol/L							
24 h postpartum	130.4	124.0	127.4	3.90	0.93	0.79	0.79
d 21 of lactation	92.0	113.0	112.0	5.71	0.27	0.16	0.42
Leucine, μmol/L							
24 h postpartum	179.6	171.5	185.4	5.34	0.70	0.70	0.46
d 21 of lactation	175.2	203.5	191.6	8.44	0.49	0.42	0.39
Lysine, μmol/L							
24 h postpartum	287.2	273.8	280.2	14.93	0.94	0.86	0.77
d 21 of lactation	73.0	88.5	132.8	11.66	0.12	0.04	0.71
Methionine, μmol/L							
24 h postpartum	98.4	95.5	91.0	3.51	0.78	0.50	0.97
d 21 of lactation	57.2	68.8	73.2	4.15	0.25	0.11	0.98
Phenylalanine, μmol/L							
24 h postpartum	98.6	83.6	89.6	37.3	0.32	0.36	0.23
d 21 of lactation	78.6	72.3	79.6	4.05	0.92	0.93	0.69
Threonine, μmol/L							
24 h postpartum	292.0	281.2	276.4	14.0	0.92	0.71	0.93
d 21 of lactation	159.8	158.3	155.8	9.09	0.98	0.89	0.89
Tryptophan, μmol/L							
24 h postpartum	79.8	78.0	81.0	2.13	0.68	0.58	0.48
d 21 of lactation	54.6	59.0	62.8	2.87	0.56	0.30	0.89
Valine, μmol/L							
24 h postpartum	250.2	264.8	257.0	9.19	0.85	0.79	0.62
d 21 of lactation	243.6	270.0	257.4	11.37	0.76	0.64	0.59
Trp/LNAA[3] ratio							
24 h postpartum	0.1039	0.1048	0.1048	0.0029	0.97	0.83	0.94
d 21 of lactation	0.0808	0.0786	0.0852	0.0039	0.77	0.66	0.58

[1] Blood samples from 4 sows per treatment.
[2] Standard error of mean
[3] Large neutral amino acid (sum of Ile, Leu, Val, Phe, and Tyr).

Table 19. Effect of dietary tryptophan levels in lactation on milk composition and estimated nutrient output of sow milk

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Milk composition							
Casein, %							
24 h postpartum	7.95	7.74	6.03	0.575	0.45	0.26	0.60
d 21 of lactation	4.30	4.39	4.37	0.071	0.87	0.72	0.73
Fat, %							
24 h postpartum	7.93	6.30	9.78	0.729	0.20	0.32	0.13
d 21 of lactation	7.52	7.34	7.06	0.237	0.72	0.45	0.83
Protein, %							
24 h postpartum	10.28	9.93	7.39	0.840	0.43	0.24	0.60
d 21 of lactation	4.52	4.53	4.61	0.061	0.70	0.45	0.76
Lactose, %							
24 h postpartum	3.68	3.89	4.43	0.161	0.22	0.10	0.65
d 21 of lactation	6.30	6.37	6.33	0.035	0.78	0.74	0.55
Total solid, %							
24 h postpartum	25.06	23.00	24.02	0.971	0.77	0.72	0.54
d 21 of lactation	19.56	19.50	19.22	0.241	0.85	0.61	0.85
Solid not fat, %							
24 h postpartum	14.54	14.50	12.18	0.709	0.42	0.26	0.52
d 21 of lactation	11.04	11.16	11.17	0..092	0.81	0.56	0.80
Free fatty acid, %							
24 h postpartum	5.24	4.99	4.51	0.150	0.19	0.08	0.73
d 21 of lactation	4.50	4.70	4.33	0.134	0.64	0.67	0.41
Estimated nutrient output of sow milk[3], piglet/day							
Milk production[4], g	697.7	697.7	718.4	13.45	0.74	0.51	0.70
Milk dry matter[5], g	129.8	129.8	133.2	2.16	0.74	0.51	0.70
Milk energy[6], kcal	850.2	850.2	871.4	13.67	0.74	0.18	0.51

[1] Milk samples from 4 sows per treatment.
[2] Standard error of mean.
[3] Noblet and Etienne, 1989.
[4] Milk production (g/piglet/day)=2.50*ADG (piglet average daily gain, g) + 80.2*BW_i (piglet BW at the beginning, kg) + 7.
[5] Milk dry matter (g/piglet/day)=0.40*ADG + 12.9*BW_i + 19.
[6] Milk energy (kcal/piglet/day)=2.54*ADG + 78.7*BW_i + 153.

Table 20. Effect of dietary tryptophan levels in lactation on protein, fat, and energy contents and their changes in lactating sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Protein[3], kg							
24 h postpartum	35.21	35.18	35.73	0.515	0.88	0.68	0.79
d 21 of lactation	35.03	34.74	35.02	0.529	0.97	0.99	0.81
Changes (d 0 - 21)	-0.18	-0.44	-0.71	0.166	0.39	0.18	0.92
Fat[4], kg							
24 h postpartum	51.21	50.52	54.41	1.481	0.97	0.96	0.80
d 21 of lactation	50.01	47.58	49.85	1.461	0.69	0.96	0.40
Changes (d 0 - 21)	-1.20	-2.94	-1.56	0.781	0.66	0.85	0.37
Energy[5], MJ							
24 h postpartum	2956.10	2926.92	2978.04	65.452	0.95	0.90	0.78
d 21 of lactation	2902.66	2795.11	2895.43	66.625	0.73	0.96	0.43
Changes (d 0 - 21)	-53.44	-131.81	-82.61	34.782	0.68	0.75	0.42

[1] Dourmad et al., 2008.
[2] Standard error of mean
[3] Protein content of sow = 2.28 + 0.178*empty body weight (0.96*BW, kg) − 0.333*BF thickness (at P_2 site, mm).
[4] Fat content of sow = −26.4 + 0.221* empty body weight + 1.331*BF thickness.
[5] Energy content of sow = −1074 + 13.65* empty body weight + 45.94*BF thickness.

Chapter Ⅴ. Effects of Dietary Tryptophan Levels in Gestating Sows on Nutrient and Amino Acids Digestibility

Abstract

This experiment was conducted to evaluate the effect of dietary Trp levels on nutrient digestibility in gestating sows. A total of 9 multiparous gestating sows (Yorkshire × Landrace) with an initial BW of 255.84 ± 20.84kg was used in a digestibility trial. Sows were allotted to one of three treatments in a completely randomized design by their BW, BF thickness, and their parity. Each treatment was designed based on relative ratio Trp to Lys in the gestation diet. Treatments were as followed: 1) Trp18%: 18% *ai* Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. An experimental diet contained 2,333 kcal NE/kg, 12.00% CP, 0.501% *ai* Lys, 68% *ai* TSAA/*ai* Lys, and 72% *ai* Thr/*ai* Lys, as fed basis. All other nutrients in experimental diet were met or exceeded the NRC requirement (2012). Each treatment diet was provided 2,400 g/d once daily to gestating sows. There were no differences in BW, BF thickness, and their changes during digestibility trial. In nutrient digestibility, dietary Trp levels showed no effect on digestibility of DM, CP, fat and ash (P=0.73, P=0.98, P=0.41, and P=0.13, respectively). In addition, there was no significant difference in nitrogen retention. Except Trp and Ile, dietary *ai* Trp/*ai* Lys level over 18% had no effect on the other amino acids digestibility. Trp digestibility was improved linearly (P=0.04) as *ai* Trp/*ai* Lys level was increased and Trp digestibility of Trp26% treatment was higher than those in Trp18% and Trp22% treatments (P=0.09) whereas, digestibility of Ile was decreased (linear, P=0.05). Although Ile digestibility was reduced by increasing dietary Trp level, this experiment represented that nutrient digestibility and nitrogen retention were not different when sows were fed diets containing *ai* Trp/*ai* Lys above 18%.

Key words: Gestating sow, Tryptophan, Nutrient digestibility, Amino acid, Nitrogen retention

Introduction

Dietary tryptophan (Trp) had several effects on pigs. Increasing dietary Trp level improved growth performance and protein deposition (Meunier-Salaun et al., 1991; Henry, 1995; Koopmans et al., 2005). Also, it regulated the appetite of pig and increased feed intake (Bubenik et al., 1996; Inui et al., 2004; Sakata and Sakai, 2010). Additional Trp supplementation reduced stress response (Bosch et al., 2007; McCloskey et al., 2009) and improved immune responses for health maintenance (Moffet and Namboodiri, 2003; Le Floch and Seve, 2007). Since these effects of dietary Trp, supplementation of Trp could have positive effect on gestating sows under stall housing.

Several swine nutrition research centers have suggested the Trp requirement in gestating sows (CVB, 2007; KSU, 2007; NRC, 2012; DPP, 2012). During the gestation, Trp requirement range (digestible Trp/Lys) in sows was huge about 15% ~ 30%. Danish nutrient standard (2015) showed the highest digestible Trp/Lys ratio (30%), CVB (2007) was the lowest ratio (15%). There were several researches, which were studied to be determined essential amino acids requirement (Lys, Met, Thr, and Trp) at early, middle, and late gestation (Srichana, 2006; Samuel et al., 2010, Levesque et al., 2011; Moehn et al., 2012). However, the research of Trp digestibility in pigs was limited. Burgoon et al. (1992) reported that high dietary Trp level increased Trp digestibility and decreased digestibility of leucine and phenylalanine in starting pig. Also, high dietary Trp level increased Trp digestibility of growing pig and improved digestibility of histidine, lysine, and Trp in finishing pig. In addition, dietary Trp level did not affect apparent digestibility of gross energy and nitrogen. Unfortunately, apparent digestibility of dietary Trp level in gestating sows was not conducted until these days.

Therefore, this experiment was conducted to evaluate the effect of dietary Trp levels on nutrient digestibility and other AAs availability in gestating sows and find relationships between dietary Trp level and its effects of gestating sows through digestibility of Trp and other nutrients.

Materials and Methods

Animal and housing

A total of 9 gestating sows (Yorkshire × Landrace, d 90 of gestation) with initial BW of 255.84 ± 20.84 kg, BF thickness (P_2 position) of 23.6 mm and average parity 6.7 was used in a digestibility trial. Sows were allotted to 1 of 3 treatments in a completely randomized design by their body weight, backfat thickness and their parity. Each treatment diet was provided 2,400 g/d once daily to gestating sows. Water is provided to sows *ad libitum*. Sows were moved into individual farrowing crates (2.5m × 1.8m) for digestibility trial and housed until end of digestibility trial. After 3days of adaptation period (87-89d gestation), sows were fed the experimental diet to each treatment during 3 days (90-92d gestation) with collecting the feces samples during 3-5 days (93-97d gestation). BW and BF thickness of sows were measured at d 90 of gestation and end of collection.

Treatment and experimental diet

Each treatment was designed based on relative ratio Trp to Lys in the gestation diet. Treatments were as followed: 1) Trp18%: 18% *ai* (apparent ileal digestible) Trp/*ai* Lys of diet, 2) Trp22%: 22% *ai* Trp/*ai* Lys of diet, and 3) Trp26%: 26% *ai* Trp/*ai* Lys of diet. An experimental diet (gestating sows' diet) contained 2,333 kcal NE/kg, 12.00% crude protein, 0.501% *ai* Lys, 68% *ai* TSAA/*ai* Lys, and 72% *ai* Thr/*ai* Lys as fed basis. All nutrients were met or exceeded NRC (2012) nutrient requirement. The formula and chemical composition of experimental diets were presented in Table 21.

Sample collection and analysis

Total collection method was used for the apparent nutrient digestibility. After a 5 day adaptation period, 3 days of feeding period and 3days of collection period were followed. Total amount of feed consumed was recorded daily for 3 days and weight of collected excreta was recorded daily for 3-5 days after feeding the experimental diet. After 3days of feeding experiment diet period, sows were fed same treatment diet for minimizing the variation of digestibility. Each treatment diet was provided 2,400 g/d once daily to gestating

sows and water also provided freely. Ferric oxide and chromium oxide were used as indigestible markers. To determine the first and last day of collection days, 0.5% of ferric oxide added in the first experimental diet and 0.5% of chromium oxide added in the fourth experimental diet (after 3days of feeding time) as selection marker, respectively. Collected excreta from each sow were pooled, sealed in plastic bags and kept frozen at -20℃. When end of the collecting excreta, total amount of excreta was weighted by weighing machine (CAS, Korea) and restored in freezer at -20℃ until further analysis. Then, after air-forced oven dried at 60℃ for 72 h and weighed those samples, those samples were ground by a Wiley mill to pass 1 mm screen for chemical analysis. Ground diets and fecal samples were analyzed for dry matter (DM) (967.03; AOAC, 1995); crude ash (923.03; AOAC, 1995); ether extract (920.39; AOAC, 1995), nitrogen by using the Kjeldahl procedure with Kjeltec (KjeltecTM 2200, Foss Tecator, Sweden) and calculated CP content (Nitrogen × 6.25; 981.10; AOAC, 1995). Also, the amino acid content of feed and feces samples were analyzed by 2-Ninhydrin method. Total urine was collected daily in a plastic container containing 50 ml of 4 N H_2SO_4 to avoid nitrogen evaporation and frozen during the 3 days of collection period for nitrogen retention analysis. Collected urine samples were stored –20℃ until analyzed. The nitrogen content of urine was calculated from CP content (Nitrogen × 6.25; 981.10; AOAC, 1995) by using the Kjeldahl procedure with Kjeltec (KjeltecTM 2200, Foss Tecator, Sweden). Considering the analyzed data and DM digestibility, digestibility of dry matter, crude protein, crude fat, crude ash and amino acid were calculated.

<Calculation>

1. Nutrient digestibility, % (DM basis)

$$\text{Digestibility (\%)} = \frac{\text{Nutrient intake} - \text{nutrient in feces}}{\text{Nutrient intake}} \times 100$$

2. Nitrogen retention, g/d

Nitrogen retention (g/d) = N intake (g) – Fecal N (g) – urinary N (g).

Statistical analysis

All statistical analyses by least squares mean comparisons were carry out using the PDIFF option with the General Linear Model procedure of SAS (SAS Institute, 2004). Orthogonal polynomial contrasts were used to determine linear and quadratic effects by increasing Trp levels in growth performance, nutrient digestibility and nitrogen retention. Individual sows were used as the experimental unit in digestibility trial and each total collected sample was used as the experimental unit in digestibility analysis. Probability values less than 0.05 ($P<0.05$) were considered as significant difference; $0.05 \leq P < 0.10$ were indicative of a trend; and values equal to or greater than 0.10 were considered as non-significant difference.

Results and Discussion

The effects of Trp supplementation level on body weight and backfat thickness of gestating sows during digestibility trail were presented in Table 22. There was no significant difference in BW at initial and final of digestibility trial and BF thickness had no significant affected by dietary Trp level during digestibility trial.

Nutrient digestibility (dry matter, crude protein, crude fat, and crude ash) and nitrogen retention of gestating sows were presented in Table 23. Dietary Trp level did not affect the digestibility of dry matter, crude protein and crude fat. Burgoon et al. (1992) reported that dietary Trp level (0.08%, 0.16%) did not affect apparent digestibility of gross energy, nitrogen in growing pigs. They also demonstrated that different dietary Trp level (0.063%, 0.143%) had no effect on apparent digestibility of gross energy and nitrogen in finishing pigs. In accordance with result of Burgoon et al. (1992), digestibility of crude protein and crude fat was unaffected by the addition of Trp in the present study. Because the nutrient contents of each experiment diet (Trp18%, Trp22%, and Trp26%) were fixed for a certain nutrient requirement of gestating sows (NE: 2,333 kcal/kg, CP: 12.0%, *ai* Lys: 0.501%, Ca: 0.7%, P: 0.4%). So, sows were fed same content of dietary energy, protein, fat, vitamins and minerals except for *ai* Trp/*ai* Lys ratio. For these reasons, different dietary Trp supplementation did not affect digestibility of dry matter, crude fat, and crude protein

significantly.

Although there was no significant difference in digestibility of crude ash, digestibility of crude ash had a tendency to increase linearly as dietary Trp level increase (linear, *P=0*.07). This result indicated that additional dietary Trp (from 18% *ai* Trp/*ai* Lys to 26% *ai* Trp/*ai* Lys) improved the crude ash digestibility (26.73%, 36.57%, 38.80%). In otherwise, Trp metabolic pathways after protein synthesis, which was known kynurenine pathway is related with immune response regulation (Moffer and Namboodiri 2003; Le Floch and Seve, 2007). Christmas et al. (2011) suggested that malnutrition and pro-inflammatory situation may result in Trp depletion thus affecting the weight gain and nitrogen balance in neonatal pigs. Trp requirement depends upon sanitary condition or heath condition. Because of Trp availability owing to sanitary condition or sows' health condition, higher dietary Trp level made the requirement of vitamins and minerals for related metabolic pathway high and digestibility of crude ash increased, too.

There was no significant difference in nitrogen retention among treatments. Meisinger and Speer (1979) reported that high dietary Trp decreased urea nitrogen and increased nitrogen retention. Since Trp is an essential component of body muscle tissue (Sidransky, 1985) and 4th limiting amino acid for protein synthesis, additional Trp supplementation improved protein deposition (Koopmans et al., 2005). Unlike previous result, current study did not show difference in nitrogen retention. NRC (2012) suggested the Trp requirement (ratio to lysine) 18% (<90d) and 20 % (>90d) in gestating sows. Libal et al. (2007) demonstrated that optimal Trp level in the diet caused less amino acids catabolism. Considering those studies, dietary Trp supplementation level (over 18% *ai* Trp/*ai* Lys) is required to balance the amino acid catabolism and it did not show difference through nitrogen retention.

In apparent AAs digestibility (Table 24), dietary Trp levels had no effect on digestibility of total AAs, aspartate, threonine, serine, glutamate, glycine, alanine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, proline, methionine, and cysteine. Trp 26% treatment had a tendency of higher Trp digestibility than other treatment (*P=0.08*) and Trp digestibility also increased linearly by dietary Trp levels (linear, *P=0.04*). Conversely, Trp 18% treatment had higher Ile digestibility than other treatments (*P*=0.09) and Ile digestibility also decreased as dietary Trp levels increased (linear, *P*=0.05). Burgoon et al. (1992)

reported that increasing dietary Trp level improved apparent Trp digestibility in starting pig (up to 11%), growing pigs (up to 8%), and finishing pigs (up to 15%). Furthermore, increasing dietary Trp level reduced the apparent digestibility of leucine and phenylalanine in starting pigs. However, high dietary Trp level (0.063% vs. 0.143%) increased apparent digestibility of histidine (74% vs. 80%) and lysine (78% vs. 83%) in finishing pigs. Trp competed with other LNAA for transportation through gastro-intestine tract and blood brain barrier (Heine et al., 1995). Increasing dietary Trp level suppressed the LNAA's retention through same metabolic pathway including isoleucine, leucine, valine, tyrosine and phenylalanine. In accordance with the result of Burgoon et al. (1992), the present study showed that increasing dietary Trp level reduced the Ile digestibility and improved the Trp digestibility linearly. Although methionine and threonine also demonstrated the negative effect on Trp uptake (Sainio et al., 1996; Sève, 1999), this study did not show effect on other amino acids digestibility by dietary Trp levels of gestation diet.

Conclusion

Trp digestibility was increased as higher level of *ai* Trp/*ai* Lys level in gestation diet on sow. On the contrary, digestibility of Ile was decreased. In spite of increasing of Trp digestibility with reduction of Ile by dietary Trp level, this experiment represented that nutrient digestibility and nitrogen retention did not show differences when sows were fed diets containing *ai* Trp/*ai* Lys above 18%.

Table 21. Formula and chemical composition of experimental diet

Items	Treatment[1]		
	Trp18%	Trp22%	Trp26%
Ingredient, % (as fed basis)			
Corn	49.42	49.02	48.62
Wheat	20.00	20.00	20.00
Soybean meal-45%CP	5.56	5.56	5.56
Rapeseed meal	4.00	4.00	4.00
Wheat bran	7.32	7.40	7.46
Soy hull	6.50	6.50	6.50
Mixed animal fat	1.08	1.20	1.30
Molasses	3.00	3.00	3.00
L-lysine·HCl	0.52	0.52	0.52
L-tryptophan 10%[2]	0.00	0.21	0.42
L-threonine 99%	0.08	0.08	0.08
Mono-di calcium phosphate	0.46	0.46	0.46
Limestone	1.21	1.20	1.21
Salt	0.35	0.35	0.35
Vitamin and mineral mix[3]	0.43	0.43	0.43
Phytase (1,000 IU)	0.07	0.07	0.07
Chemical composition (calculated values, as fed basis)			
NE, Kcal/kg	2,333	2,333	2,333
Crude protein, %	12.0	12.0	12.0
Total Lys, %	0.638	0.638	0.637
Total Trp, %	0.128	0.149	0.170
ai Lys, %	0.501	0.501	0.501
ai Trp, %	0.089	0.110	0.130
ai TSAA / *ai* Lys, %	68	68	68
ai Thr / *ai* Lys, %	72	72	72
ai Trp / *ai* Lys, %	18	22	26
Ca, %	0.7	0.7	0.7
Total P, %	0.4	0.4	0.4

[1] Trp18% (*ai* Trp/*ai* Lys, 18%), Trp22% (*ai* Trp/*ai* Lys, 22%), and Trp26% (*ai* Trp/*ai* Lys, 26%).
[2] Feed grade (CJ, Indonesia).
[3] Supplied the following per kilogram of diet: vitamin A, 15,020 IU; vitamin D_3, 3,000 IU; choline chloride, 1,200 mg; vitamin E, 80 IU; cyanocobalamine, 40 mg; niacin, 20.0 mg; Ca panthotenate, 16.0 mg; riboflavin, 4.0 mg; menadione, 4.0 mg; folic acid, 2 mg; pyridoxine, 2 mg; thiamine 0.8 mg; d-biotin, 0.5 mg; Fe, 200.0 mg (as $FeSO_4$); Mn, 79.0 mg (as $MnSO_4$); Zn, 35.0 mg (as $ZnSO_4$); Cu, 5.0 mg (as $CuSO_4\,5H_2O$); Co, 1.98 mg (as $CoSO_4$); I, 1.26 mg (as $Ca(IO_3)$); Se, 0.1 mg (as Na_2SeO_3).

Table 22. Effect of dietary tryptophan levels in gestation on body weight, backfat thickness and their changes of sows during collection period

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp 18%	Trp 22%	Trp 26%		Trp	Lin.	Quad.
Body weight, kg							
Initial	264.2	252.3	251.1	7.37	0.75	0.51	0.75
Final	268.0	256.3	255.2	7.44	0.75	0.52	0.75
Changes	3.8	4.0	4.2	0.18	0.38	0.19	1.00
Backfat thickness, mm							
Initial	24.5	23.7	22.7	2.10	0.94	0.74	0.99
Final	24.7	24.2	23.0	1.99	0.94	0.75	0.94
Changes	0.2	0.5	0.3	0.17	0.79	0.74	0.57

[1] A total of 9 gestating sows (Yorkshire × Landrace, 90d gestation) and initial BW 255.84 ± 20.84 kg.
[2] Standard error of mean.

Table 23. Effect of dietary tryptophan levels in gestation on nutrient digestibility and nitrogen retention on sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp18%	Trp22%	Trp26%		Trp	Lin.	Quad.
Nutrient digestibility, %							
Dry matter	77.65	76.30	76.97	0.518	0.73	0.70	0.52
Crude protein	76.06	75.78	75.81	0.471	0.98	0.88	0.91
Crude fat	86.53	85.85	85.09	0.363	0.41	0.21	0.96
Crude ash	26.73	36.57	38.80	2.460	0.13	0.07	0.42
Nitrogen retention							
N-intake, g/d	122.55	122.28	120.33	0.350	-	-	-
N-feces, g/d	29.34	29.62	29.11	0.576	0.96	0.91	0.82
N-urine, g/d	42.67	49.28	35.84	6.739	0.80	0.75	0.59
N-retention[3], g/d	50.54	43.38	55.37	6.290	0.82	0.81	0.58
N-retention[4], %	41.24	35.47	46.02	5.176	0.80	0.77	0.57

[1] A total of 9 gestating sows (Yorkshire×Landrace, d 90 of gestation) and initial BW 255.84±20.84 kg.
[2] Standard error of mean.
[3] N retention: N intake (g) – Fecal N (g) – urinary N (g).
[4] N retention (%): N retention / N intake×100.

Table 24. Effect of dietary tryptophan levels in gestation on apparent amino acids digestibility in sows

Items	Treatment[1]			SEM[2]	*P*-value		
	Trp 18%	Trp 22%	Trp 26%		Trp	Lin.	Quad.
Amino acids digestibility, %							
Total amino acid	83.76	87.35	83.86	1.244	0.48	0.88	0.28
Aspartate	77.70	83.27	77.80	1.910	0.48	0.91	0.28
Threonine	79.45	83.43	79.80	1.577	0.52	0.84	0.32
Serine	82.93	87.28	84.05	1.461	0.58	0.88	0.33
Glutamate	88.96	91.91	88.24	0.907	0.25	0.66	0.14
Glycine	75.81	80.68	77.59	1.916	0.73	0.87	0.46
Alanine	76.16	83.04	76.60	1.857	0.31	0.94	0.16
Valine	79.18	79.31	74.54	1.412	0.23	0.15	0.51
Isoleucine	81.26	77.91	72.93	1.710	0.09	0.05	0.99
Leucine	85.52	88.67	84.26	1.162	0.27	0.51	0.18
Tyrosine	79.39	86.00	83.10	1.692	0.40	0.48	0.27
Phenylalanine	81.32	86.91	83.71	1.655	0.53	0.70	0.31
Lysine	84.30	87.77	86.88	1.267	0.75	0.62	0.55
Histidine	94.65	96.92	96.16	0.775	0.71	0.62	0.50
Arginine	90.63	92.05	90.41	0.932	0.78	0.81	0.58
Proline	87.75	90.62	87.93	0.795	0.35	0.93	0.19
Methionine	80.36	85.10	81.52	1.701	0.61	0.88	0.36
Cysteine	86.55	87.77	87.74	1.101	0.95	0.81	0.87
Tryptophan	88.17	88.76	91.77	0.826	0.08	0.04	0.40

[1] A total of 9 gestating sows (Yorkshire × Landrace, d 90 of gestation) and initial BW 255.84 ± 20.84 kg.
[2] Standard error of mean.
[abc] means with different superscripts within the same row significantly differ ($P<0.05$).

Chapter Ⅵ. References

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Chapter Ⅶ. Overall Conclusion

In Korea, the sow reproductivity is relatively lower than that in EU countries and this trend is somewhat likely to affect the national competiveness in international pork industry like FTA. In 2010, PSY of Korea was 21.9, much lower than that of Dutch (PSY; 27.2). Several nutritional strategies including feeding scheme, energy level, energy/Lys ratio and amino acids balance in sow diets have been developed to improve sow reproductivity. As one of essential AAs, Trp was metabolized in 3 different pathways, which are protein synthesis for protein deposition, kynurenine synthesis for immune response regulation, and serotonin synthesis for feed intake and stress reducer through the hydroxylase pathway. Consequently, three experiments were conducted 1) to investigate the dietary Trp levels in sow's diet on reproductive performance in gestating and lactating sows, 2) to determine the optimal relative Trp level to Lys in gestating and lactating sows, and 3) to evaluate the nutrient digestibility in gestating sows by varying Trp/Lys ratios.

In the first study, dietary Trp levels (*ai* Trp/*ai* Lys, 18%, 22% and 26%; *ai* Lys, 0.50%) were fed to gestating sows. From insemination to weaning of sow, there were no significant differences in BW, BF thickness, and their changes by dietary Trp treatments. Furthermore, dietary Trp levels in gestating sows did not affect ADFI, WEI, and litter performance of their progeny after cross-fostering. During gestation, higher Trp level in gestation diet increased Trp level and Trp/ LNAA ratio in blood plasma significantly. Although Trp and Trp/LNAA ratio in diet were higher, there were no significant differences on blood cortisol, melatonin and serotonin. Above 22% Trp/Lys ratio in gestation diet showed the higher feed consumption until d 12 post-parturition. However, there was no positive reproductive performance derived from high feed intake during early lactation. These results indicated that highly supplementation level of Trp (22~26%) over requirement of gestating sows had no additional effects on reproductivity and optimum range of daily *ai* Trp intake during whole gestation was 1.98 g - 2.16 g (*ai* Trp/*ai* Lys, 18%; *ai* Lys, 0.50%).

The second experiment was conducted to evaluate the dietary Trp levels (*ai* Trp/*ai* Lys, 18%, 22% and 26%; *ai* Lys, 0.65%) in lactating sow's diet on reproductivity. Among

treatments, there were no differences significantly in BW, BF thickness, ADFI, and WEI. At farrowing of sows, dietary Trp levels did not affect number of piglets in total born, still born, mummy and born alive. Above 22% Trp/Lys ratio in lactation diet tended to increase litter weight and litter weight gain. And high Trp level in lactation diet showed significant difference in serotonin concentration at 24 h postpartum. Blood cortisol tended to decrease whereas blood melatonin was numerically increased as dietary Trp level of lactation diet was increased. Piglet weight, weight gain and BUN during lactation were not affected by dietary Trp level. Although blood Trp concentration increased numerically as Trp level increase in lactation feed, there was no linear or quadratic response by the effect of dietary Trp levels in Trp/LNAA. Above 22% Trp/Lys ratio in lactation diet, ADFI tended to increase in middle of lactation (d 8 - 14) numerically. In brief, high level of Trp in lactation diet improved the litter performance with reducing the delivery stress in farrowing sows. However except some criteria, excess supplementation of Trp (>18% *ai* Trp/*ai* Lys) had plateau effects to reproductivity of sows. These results indicated that the optimum ratio of *ai* Trp/*ai* Lys in lactation feed was 18%, when *ai* Lys content in lactation feed was 0.65% (as fed basis).

The third study was conducted to evaluate the effect of dietary Trp levels (*ai* Trp/*ai* Lys, 18%, 22% and 26%; *ai* Lys, 0.50%) on nutrient digestibility in gestating sows. There were no significant differences in BW, BF thickness, and their changes during digestibility trial. In nutrient digestibility, dietary Trp levels had no effect on digestibility of dry matter, crude protein, fat and ash. Trp digestibility tended to increase linearly as high dietary Trp level and high dietary *ai* Trp/*ai* Lys level reduced the digestibility of Ile. Consequently, dietary *ai* Trp/*ai* Lys level over 18% increased Trp digestibility while it diminished the Ile digestibility and did not affect nutrient digestibility and nitrogen retention in gestating sows.

In summary, dietary Trp level in sows might change Trp metabolism with LNAA metabolism and reproductive performance of sows. Some parameters of reproductivity, such as litter gain of whole lactation and daily feed intake during early-middle lactation, were improved by higher Trp level. However, over 18% *ai* Trp/*ai* Lys in sow diet is not recommendable due to the fact that beneficial effects for reproductivity of sows and low coherence with other parameters were not observed. Further studies would be required to demonstrate effects of lower dietary Trp levels than those in current studies for optimal dosage in sows.

Chapter Ⅷ. Summary in Korean

본 연구는 1) 임신돈 및 포유돈 사료 내 다양한 수준의 트립토판이 번식성적에 미치는 영향, 2) 임신돈 및 포유돈에 있어서 라이신 대비 적정 트립토판 비율, 3) 다양한 수준의 트립토판이 영양소 및 아미노산 소화율에 미치는 영향을 규명하기 위하여 수행되었다.

Experiment Ⅰ. Effects of Dietary Tryptophan Levels in Gestating Sows on Performance and Blood Metabolites

본 실험은 임신돈 사료 내 다양한 수준의 트립토판이 번식성적에 미치는 영향을 규명하기 위하여 수행되었다. 총 45두의 F1 경산돈 (Yorkshire × Landrace)을 공시하여, 완전임의배치법에 의하여 3처리, 처리당 15두로 교배 후의 체중, 등지방 두께를 고려하여 배치하였다 (평균 체중, 218.96±2.43 kg; 평균 산차, 4.8±0.03). 전체 실험군은 교배 후 21일차에 재발정을 확인하였으며, 초음파 진단기를 사용하여 35일에 임신 진단을 하였다.

임신돈의 일일 사료 급여량은 2산차에는 2.2, 3산차에는 2.4kg을 각각 급여하였고, 분만 시점을 기준으로 전후 5일간은 번식돈 급여프로그램에 따라서 제한급여를 실시하고, 이후 이유 시점까지는 포유돈 사료를 자유 급여하였다. 각 처리구는 임신돈 사료의 트립토판의 수준에 따라서 설계되었다. 외관상회장 가소화율을 기준으로 실험 사료 내 라이신 대비 트립토판의 비율 (18%, 22%, 26%)에 따라서 Trp18%, Trp22%, Trp26%로 나누었다. 임신 기간 동안에는 각 처리에 따라서 임신돈 실험 사료를 급여하였으며, 분만 전 5일부터는 일반 포유돈 사료가 동일하게 급여되었다. 임신돈 실험 사료는 정미 에너지 2,333 kcal/kg, 조단백질 12.0%, 외관상회장가소화 라이신 0.501%으로 설계되었다. 외관상회장가소화율을 기준으로 트립토판을 제외한 황함유아미노산 및 트레오닌의 함량은 라이신 대비 각각 68%, 72%였다. 본 실험 결과, 체중, 등지방

변화, 이유 후 재귀발정일 및 포유 기간 중 평균 일일 사료 섭취량에서 유의적인 차이가 발생하지 않았다. Trp18% 처리구에서 총 산자수 (P=0.07) 및 생존 산자수 (P=0.06)가 다른 처리구에 비하여 높게 나타났으나, 사산돈 및 미이라는 유의적 차이를 보이지 않았다. 양자 처리 후의 포유 성적에서도 복당 체중, 복당 증체량 및 포유 자돈 일일 증체에서 유의적인 차이가 나타나지 않았다. 임신 35일령에 Trp22% 처리구에서 혈중 요소태 질소 농도가 유의적으로 높았으며 (P=0.03), 혈중 콜티졸 농도가 다른 처리구에 비하여 낮은 경향을 보였다 (P=0.06). 임신 35일령의 혈중 멜라토닌 농도는 사료 내 트립토판 수준이 증가할수록 유의적으로 증가하였다 (P=0.02). 혈중 세로토닌의 농도는 실험 전 기간 동안, 처리구별 유의적인 차이를 보이지 않았다. 임신기 동안, 혈중 트립토판 농도 및 혈중 방향족 아미노산 농도 대비 혈중 트립토판 농도 비율은 실험 사료 내 트립토판이 증가함에 따라서 선형적으로 증가하는 결과를 보였지만 (P<=0.01), 해당 기간 동안 혈중 콜티졸, 멜라토닌 및 세로토닌의 농도는 선형적인 변화를 나타내지 않았다. Trp26% 처리구에서 유의적으로 임신 35일, 70일 및 110일에서 Trp18% 처리구 대비 높은 수준의 혈중 트립토판 농도를 보였다 (P<0.01; P=0.03; P<0.01). 초유 및 이유 시점의 모유에서 처리구간의 유 생산량 및 유 성분은 유의적인 차이를 보이지 않았다. 포유 10일 ~ 11일차에 Trp22% 및 26% 처리구는 Trp18%보다 유의적으로 일일사료섭취량이 높았으나 (P<0.05), 실험 전 기간 동안 모돈의 체내 단백질, 지방, 에너지 함량 및 기간별 변화량에 있어서는 유의적인 차이가 나타나지 않았다. 본 실험 결과, 임신 기간 내 다른 수준의 트립토판은 임신 초기 사료 섭취량에만 영향을 미쳤을 뿐, 기타 번식 성적에는 유의적인 차이가 없었다. 다만, 혈액 성상에 있어서는 임신 기간 중에 사료 내의 트립토판 함량에 비례하여, 혈중 트립토판 농도 및 방향족 아미노산 대비 트립토판의 비율이 유의적으로 증가하였으나, 그에 따른 콜티졸, 세로토닌 및 멜라토닌의 변화는 숫자적으로 감소는 했으나 유의적인 차이는 나타나지 않았다. 임신돈 사료 내 라이신 대비 트립토판의 비율이 22% 이상일 경우에는 임신 기간 동안 혈중 트립토판의 증가 및 포유 초기 사료 섭취량 증대 효과가 있으나, 그로 인한

번식 성적 개선 효과는 나타나지 않았다. 그에 따라서, 임신돈 사료 내 트립토판의 첨가 수준이 라이신 대비 22% 이상에서는 추가적인 번식 성적 개선이 나타나지 않았으며, 임신돈 사료 내 적정 트립토판의 수준은 라이신 대비 최대 18% 수준이라고 사료된다. 본 실험에서의 임신기 동안 급여 프로그램 및 사료 내 라이신(0.501%) 대비 18% 수준의 트립토판 수준을 고려하였을 때, 임신기 전 기간 동안 일일 최대 트립토판 섭취량은 1.98 g ~ 2.16 g 수준으로 판단된다.

Experiment Ⅱ. Effects of Dietary Tryptophan Levels in Lactating Sows on Performance and Blood Metabolites

본 실험은 포유돈 사료 내 다양한 수준의 트립토판 수준이 번식 성적에 미치는 영향을 조사하기 위해서 수행하였다. 총 30두의 F1 경산돈 (Yorkshire × Landrace) 을 공시하여, 완전임의배치법에 의하여 3처리, 처리당 10두로 체중, 등지방 두께를 고려하여 배치하였다 (평균 체중, 234.27±17.35 kg; 평균 산차, 4.73). 분만 시점 전후 5일간은 번식돈 급여프로그램에 따라서 제한 급여를 실시하고, 이후 이유 시점까지는 포유돈 사료를 자유 급여하였다. 각 처리구는 포유돈 사료의 트립토판의 수준에 따라서 설계되었다. 외관상회장가소화율을 기준으로 실험 사료 내 라이신 대비 트립토판의 비율 (18%, 22%, 26%)에 따라서 Trp18%, Trp22%, Trp26%로 나누었다. 포유돈 실험 사료는 정미 에너지 2,350 kcal/kg, 조단백질 13.5%, 외관상회장가소화 라이신 0.650%으로 설계되었다. 외관상회장가소화율을 기준으로 트립토판을 제외한 황함유아미노산 및 트레오닌의 함량은 라이신 대비 각각 62%, 75%였다. 포유 기간 중의 체중, 등지방 및 각각의 변화, 그리고 이유 후 재발정일에 있어서, 포유돈 사료 내 트립토판의 수준에 따른 유의적인 차이는 나타나지 않았다. 또한 분만시, 번식 성적 (총 산자수, 생존 산자수, 사산돈, 그리고 미이라)에 있어서는 유의적인 차이를 나타내지 않았다. 트립토판 첨가 수준이 높아질수록, Trp18% 처리구 대비

포유 기간 중, 복당 체중 (P=0.06). 및 복당 증체 (P=0.04)에서 유의적으로 높은 경향을 나타내었다. 하지만, 포유 자돈의 개체별 일당증체량은 처리에 따른 유의적 차이가 없었다. 또한 혈중 요소태 질소, 콜티졸, 그리고 멜라토닌 농도에서는 포유돈 사료 내 트립토판 수준에 따른 유의적 차이가 없었으나, 라이신 대비 트립토판의 수준이 22% 이상일 경우에는 혈중 세로토닌의 수준이 18% 대비 유의적으로 높게 나타났다 (P=0.03). 하지만, 동일 시점인 분만 후 24시간 이후의 혈중 콜티졸 농도는 유의적인 차이를 나타나지 않으며, 숫자적으로 낮아지는 경향을 보였다 (P=0.02). 포유돈 사료 내 트립토판 수준이 높아짐에 따라서, 혈중 트립토판 농도가 높아지는 경향을 보였으나, 방향족 아미노산 대비 트립토판의 농도 비율은 유의적인 차이를 보이지 않았다. 추가적으로, 초유 및 이유 시점의 돈유에서 유 생산량 및 유 성분에서 역시 각 처리에 따른 유의적 차이를 보이지 않았다. 하지만, 사료 내 라이신 대비 트립토판의 비율이 22% 이상일 경우에는 포유 8일령부터 14일령까지 일일섭취량이 증가하는 경향을 보였으나, 유의적인 차이는 없었다. 본 실험 결과, 포유돈 사료 내 라이신 대비 트립토판의 비율이 22% 이상일 경우에는 번식 성적 개선이 없었으나 22% 수준에서는 포유 초기 섭취량 개선 및 분만 스트레스를 감소할 혈중 세로토닌의 함량이 증가함을 나타내었다. 하지만 이러한 개선이 번식 성적의 개선으로 나타나지 않았기에, 외관상회장가소화 라이신 대비 트립토판의 최대 수준은 18% 수준으로 사료된다.

Experiment Ⅲ. Effects of Dietary Tryptophan Levels in Gestating Sows on Nutrient and Amino Acids Digestibility

본 실험은 번식돈 사료 내 다른 수준의 트립토판 함량이 영양소 소화율 및 아미노산 이용률에 미치는 영향을 조사하기 위해서 수행되었다. 총 9두의 F1 경산돈 (Yorkshire × Landrace) 을 공시하여, 완전임의배치법에 의하여 3처리, 처리당 3두로 체중, 등지방 두께 그리고 산차를 고려하여 배치하였다 (평균 체중,

255.84±17.35 kg). 일일 사료 급여량은 2.4 kg였으며, 각 처리구는 임신돈 사료의 트립토판의 수준에 따라서 설계되었다. 외관상회장가소화율을 기준으로 실험 사료 내 라이신 대비 트립토판의 비율 (18%, 22%, 26%)에 따라서 Trp18%, Trp22%, Trp26%로 나누었다. 소화율 실험 기간 동안, 체중 및 등지방 두께 그리고 각 항목의 변화는 유의적인 차이를 나타내지 않았다. 건물, 조단백질 및 조지방 소화율은 처리에 따른 유의적인 차이를 보이지 않았으며, 조회분의 소화율은 트립토판의 수준이 증가함에 따라서 선형적으로 증가하는 경향을 보였다 (P=0.07). 질소 축적에 대해서도 유의적인 차이를 보이지 않았다. 트립토판 및 아이소루신을 제외하고는 처리에 따른 아미노산의 소화율은 유의적 차이가 없었다. 사료 내 트립토판의 수준이 증가할수록 트핍토판의 소화율은 증가하였으며 (P=0.04), 아이소루신 (P=0.05)의 소화율은 선형적으로 감소하였다. 본 실험 결과, 임신돈 사료 내 트립토판의 수준이 증가할수록, 사료 내 트립토판의 소화율이 높아짐과 동시에 아이소루신의 소화율은 감소하였으나, 질소 이용성에 있어서는 차이가 없었다.

종합적으로, 사료 내 다양한 수준의 트립토판의 수준은 트립토판의 대사 및 LNAA의 대사에 영향을 미치며, 복당 증체량 및 포유 초기 섭취량 등에 영향을 미치는 것으로 나타났다. 하지만, 임신 및 포유 기간 중, 라이신 대비 트립토판의 수준이 22% 이상일 때, 18% 대비 번식 성적에 긍정적인 영향이 나타나지 않았으며, 트립토판의 대사 변화와 관련된 기타 분석 항목에서는 연관성이 낮게 나타났다. 그에 따라서, 사료 내 라이신 대비 트립토판의 함량을 18% 이상 급여하는 것은 번식 성적 개선이 없을 것으로 사료된다 (임신돈 사료 내 라이신 함량; 0.50%, 포유돈 사료 내 라이신 함량; 0.65%). 추가적으로 모돈 사료 내 적정 트립토판 수준을 찾기 위해서는 라이신 대비 트립토판의 비율이 본 실험보다 낮은 18% 미만의 수준에서의 추가 실험이 필요하다.