ผลของการเสริมแอล-ทริปโตเฟนระดับสูงในช่วงสั้นต่อพฤติกรรมความเครียด และลักษณะซากของสุกร

เกชา คูหา[1*] ประมวล เติมสมบัติถาวร[2] และ สุวรรณ ช่างกลึงดี[3]
มหาวิทยาลัยเทคโนโลยีราชมงคลล้านนา น่าน อ.ภูเพียง จ.น่าน 55000 ประเทศไทย

บทคัดย่อ

กรดอะมิโนทริปโตเฟนมีบทบาทสำคัญในการเป็นสารตั้งต้นสำหรับสังเคราะห์สารเซอโรโทนิน ซึ่งมีผลต่อการควบคุมของสมองในส่วนที่เกี่ยวข้องกับการแสดงออกเชิงพฤติกรรมและการควบคุมกลไกทางสรีระที่สำคัญ เช่น การแสดงออกทางอารมณ์ ความก้าวร้าว ความไวต่อความเครียด รูปแบบการนอนหลับพักผ่อน และการกินอาหาร และเนื่องจากการเลี้ยงสุกรในปัจจุบันให้ความสำคัญต่อระบบการผลิตที่ดี โดยคำนึงถึงความเป็นอยู่ของสุกรที่สุขสบายและมีสวัสดิภาพ จึงคาดว่าการเสริมแอล-ทริปโทเฟนในอาหารระดับสูงจะช่วยลดสภาวะเครียดของการจัดการฝูงสุกร อันจะส่งผลดีต่อประสิทธิภาพการเลี้ยงและคุณภาพซากได้ อย่างไรก็ตามในประเทศไทยไม่พบว่ามีข้อมูลการวิจัยเกี่ยวกับการใช้ทริปโตเฟนเพื่อช่วยลดพฤติกรรมก้าวร้าว ลดความเครียด และเพิ่มคุณภาพซากของสุกร งานวิจัยนี้จึงศึกษาการเสริมแอล-ทริปโตเฟนระดับสูงในอาหารสุกรในช่วงการเลี้ยงต่างๆ เพื่อศึกษาถึงพฤติกรรมโดยทั่วไป พฤติกรรมก้าวร้าว ค่าบ่งชี้ความเครียด และคุณภาพเนื้อสุกร โดยจัดทรีทเมนต์ควบคุมเป็นการให้อาหารมีทริปโตเฟนในระดับความต้องการปกติเพื่อการเจริญเติบโต ส่วนทรีทเมนต์ที่ 2 และ 3 ทำการเสริมแอล-ทริปโตเฟนระดับสูงขนาด 2 เท่า และ 4 เท่าจากความต้องการปกติ ตามลำดับ จากการทดลองพบว่า สุกรแสดงพฤติกรรมโดยทั่วไปไม่แตกต่างกันทางสถิติ ในแง่ของพฤติกรรมก้าวร้าวของสุกรแม้จะพบว่าไม่มีความแตกต่างกันทางสถิติ แต่สุกรที่ได้รับการเสริมแอล-ทริปโตเฟนสูงกว่าปกติ 4 เท่าจะมีจำนวนครั้งของการกัดกันน้อยที่สุด ค่าฮอร์โมนคอร์ติซอลในน้ำลายช่วงการจัดฝูงและการขนส่งสุกรไปโรงฆ่า บ่งชี้ว่าสุกรในทุกทรีทเมนต์เกิดความเครียดสูงในช่วงดังกล่าว แต่สุกรที่กินอาหารที่มีแอล-ทริปโตเฟนระดับสูงขึ้นจะมีระดับฮอร์โมนคอร์ติซอลต่ำลง ส่วนค่าอุณหภูมิซาก 45 นาทีหลังฆ่าของสุกรที่ได้รับทริปโตเฟนระดับปกติจะค่อนข้างสูง แตกต่างจากทรีทเมนต์อื่น การเสริมแอล-ทริปโตเฟนในอาหารขนาด 4 เท่าของระดับปกติ และให้สุกรกินเป็นช่วงสั้นๆ ในช่วงความเครียดสูงจะช่วยลดความเครียดของสุกร และช่วยปรับปรุงคุณภาพของเนื้อสุกรหลังฆ่าได้

คำสำคัญ : เซอโรโทนิน / คอร์ติซอล / สวัสดิภาพ / เนื้อสุกร

* *Corresponding author; E-mail: k.kuha3@gmail.com; Tel: +66(54)-710-259 ext. 7239-40; Fax: +66(54)-771-398*

1 *ผู้ช่วยศาสตราจารย์ สาขาสัตวศาสตร์และประมง คณะวิทยาศาสตร์และเทคโนโลยีการเกษตร*

2 *รองศาสตราจารย์ สาขาสัตวศาสตร์และประมง คณะวิทยาศาสตร์และเทคโนโลยีการเกษตร*

3 *อาจารย์ สาขาสัตวศาสตร์และประมง คณะวิทยาศาสตร์และเทคโนโลยีการเกษตร*

Effects on Pig Behavior, Stress and Carcass Characteristics of a Short-term Diet with High Levels of L-tryptophan

Kecha Kuha[1*], Pramual Toemsombatthaworn[2], and Suwan Changkleungdee[3]
Rajamangala University of Technology Lanna Nan, Phu Phiang, Nan 55000 Thailand

Abstract

Tryptophan plays an important role as the immediate precursor of serotonin synthesis. The serotonin activity in the brain has been implicated in the regulation of behavioral and physiological processes such as mood, aggression, susceptibility to stress, sleep patterns and feed intake. The pig production systems of today emphasize concern with the animal's well-being and welfare. Therefore, a high supplemental L-tryptophan, through its ability to increase serotonin, may reduce stress and improve performance and pork quality. However, there is no information available for pig farms in Thailand of supplemental L-tryptophan's effect on behaviors, stress and pork quality. This work determined how three levels of dietary tryptophan, starting with the standard requirement for growth (control), going to twice (2×), and finally 4 times (4×) the control amount, affected the general and aggressive behaviors, and stress in pigs and also pork quality. The results found no difference in general behavior of pigs between the treatments. The aggressive behaviors of pigs also expressed no significant difference between the treatments. However, biting behavior was the lowest at the 4× supplemental diet. Salivary cortisol levels indicated high stress in the pigs during regrouping and transportation. Carcass temperature 45 min postmortem was the highest at the standard level tryptophan diet. These results give the evidence that a short-term supplementation of four times the requirement for growth of L-tryptophan in a diet in pigs can reduce stress during regrouping and transporting and improve meat quality after slaughter.

Keywords : Serotonin / Cortisol / Welfare / Pork

---

* Corresponding author; E-mail: k.kuha3@gmail.com; Tel: +66(54)-710-259 ext. 7239-40; Fax: +66(54)-771-398
[1] Assistant Professor, Department of Animal Science and Fisheries, Faculty of Sciences and Agricultural Technology
[2] Associate Professor, Department of Animal Science and Fisheries, Faculty of Sciences and Agricultural Technology
[3] Lecturer, Department of Animal Science and Fisheries, Faculty of Sciences and Agricultural Technology

1. Introduction

Tryptophan (Trp) is an essential amino acid for growth in pigs. It has been shown to affect the brain and nervous system through interference with serotonergic neurotransmission [1-3]. It serves as the immediate precursor for serotonin synthesis. The serotonin activity in the brain has been implicated in the regulation of behavioral and physiological processes such as mood, aggression, susceptibility to stress, immune response and feed intake [3-4]. The pig production in Thailand concerns itself with the well-being and welfare of the animals. Pig management applies to a large herd as an all-in all-out system. This means that piglets from many litters are mixed together in large pens. During production, pigs sometime need to be regrouped to conserve the uniformity of the herd. This handling can easily induce stress and is unavoidable. These stresses can occur many times during the process of pig management, most notably during mixing, regrouping, transportation and slaughter. Stress influences pigs to lose or slowly gain body weight and to be easily susceptible to disease [5-10]. Stress prior to slaughter also affects carcass quality with meat appearing pale, soft and with exudates of the muscle tissue (PSE). Many researchers have shown that adding L-tryptophan (L-Trp) to the diet may reduce aggression, alleviate stress and decrease plasma cortisol in the animals [11-13]. Consequently, supplemental L-Trp may affect the response of pigs to stress through its ability to increase serotonin and it has a sedative effect [14]. If dietary Trp is able to reduce stress, it may increase feed intake and the overall growth performance of pigs. Furthermore, in the finishing pig, Trp can improve pork quality by reducing stress during slaughtering [1-3, 9-10].

These kinds of studies have not been done for the pig industry in Thailand. Therefore, this research investigated the effect of a short-term supplemental L-tryptophan diet in the pig as a feed basis on the general and aggressive behaviors in pigs, their stress level during regrouping and transportation, and after slaughter, some of their carcass characteristics as function of pork quality.

2. Materials and methods

2.1 Trial 1

Trial 1 determined the general behavior of an early growth pig (body weight about 15-30 kg). The experimental design was a completely randomized block (RBD). The block effect was tree groups of crossbred pigs according to Y50:L50, Y25:L75 and Y25:L25:P50 percents, where Y is the Large white; L is the Landrace and P is the Piétrian. The treatment effect was tree levels of tryptophan (Trp) in the pig diet, based on the standard requirement for growth (control), twice (2×), and four times (4×) the control amount (Table 1 and 2). Sixty-three healthy pigs were randomized into nine pens of seven pigs each. Pigs were allowed to familiarize themselves with each other and the pen for seven days before starting the experiment. During the subsequent seven days, the pigs were fed the three dietary treatments. After that, all pigs were returned to the control diet for an additional seven days. General behaviors like lying, standing, walking, and eating/drinking were observed from 13.00-15.00 pm of days 3, 5, 7, 9, 11 and 13 of the experiment. Nine trained observers were randomized to the first pen and asked to observe those behaviors for 10 min. The proportions of behaviors were determined by instantaneous scan sampling of pig activity at every min after signaling. After five scans of observing, the pigs of all pens were subjected to a startling stimulus using a vibratory sound. The resulting behaviors were observed for the next five scans by the same observer and the same procedure. After that the observers were rotated to the next pen and they continued to observe for the next 10 min following the same procedure until each pen was observed for a total of 90 scans.

Table 1 The diets of the 3 trials

Components	Trial 1			Trial 2			Trial 3		
	Control	2×-Trp	4×-Trp	Control	2×-Trp	4×-Trp	Control	2×-Trp	4×-Trp
Corn	50.00	49.94	49.72	56.40	56.40	56.40	56.50	56.50	56.50
Rice bran	10.60	10.60	10.60	21.00	21.00	21.00	26.50	26.50	26.50
Soybean meal	30.00	30.00	30.00	10.00	10.00	10.00	27.50	27.50	27.50
Fish meal	36.00	35.80	35.50	14.50	14.50	14.50	23.00	23.00	23.00
Meat and bone meal	-	-	-	15.00	14.73	14.30	22.25	23.00	22.50
Lipid	31.00	31.00	21.00	10.50	10.60	10.70	20.50	20.60	20.80
Di-calcium phosphate	31.80	31.80	21.80	11.80	11.80	11.80	21.80	21.80	21.80
Salt	30.35	30.35	20.35	10.30	10.30	10.30	20.45	20.45	20.45
Premixed	30.25	30.25	20.25	10.30	10.30	10.30	20.30	30.30	20.30
L-Tryptophan	30.00	30.26	20.78	-	10.17	10.50	-	30.15	20.45
Digestive Enzyme	-	-	-	10.20	10.20	10.20	20.20	20.20	20.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 2 The chemical compositions of the 3 dietary treatments

Chemical compositions	Trial 1			Trial 2			Trial 3		
	Control	2×-Trp	4×-Trp	Control	2×-Trp	4×-Trp	Control	2×-Trp	4×-Trp
Moisture (%)	10.24	10.17	9.94	9.17	9.28	8.75	9.21	9.16	9.17
Protein (%)	21.86	22.16	22.35	16.27	16.80	16.86	13.74	13.6	14.47
Fat (%)	4.81	4.71	2.39	3.18	2.31	4.77	4.26	5.16	4.82
Ash (%)	5.61	6.1	9.81	8.25	8.09	8.12	7.30	7.53	7.54
Fiber (%)	5.05	4.88	4.41	6.16	6.72	6.95	6.48	6.45	6.21
GE (kcal/kg)	3,966.22	3,985.09	4,007.07	3,828.65	3,827.81	3,921.20	3,882.51	3,874.86	3,855.16
ME (kcal/kg)	3,244.50	3.235.61	3,218.12	3,146.23	3,147.90	3,146.02	3,137.56	3,139.68	3,143.92
Tryptophan (%)	0.26	0.51	1.00	0.17	0.33	0.64	0.15	0.29	0.57
Metionine (%)	0.40	0.40	0.39	0.33	0.32	0.32	0.29	0.28	0.28
Lysine (%)	1.33	1.32	1.30	0.89	0.88	0.87	0.73	0.73	0.72

GE is the gross energy; ME is the metabolizable energy.

2.2 Trial 2

Trial 2 was conducted to determine aggressive behaviors in growing pigs (40-60 kg) during regrouping. The design was a Latin square, to which the effect of pig size was added as another fixed effect of trial 1. Fifty-four healthy growing pigs were divided into three sizes within three breed groups. The animals, after that, were randomly paired within size. Then, the couples were put into a pen sized 1.2 × 1.5 m^2 for a total of 27 pens. After allowing the pigs a social adjustment for seven days, the three dietary treatments (Table 1 and 2) were supplied to each allotted pigpen for two weeks. Aggressive behaviors were observed on days 4, 5 and 6 of the experiment. The coupled pigs of the same breed group and size from three pens were carried to the observation pen. These 3 × 4 m^2 pens had a fully solid slat with dense walls and no other instrument inside. Aggressive behaviors were counted for 30 min using three trained observers. Behavior observation was divided into three stages every 10 min. The behaviors were classified according to the procedure described by Ruis [15]. To identify stress by examining salivary cortisol, saliva specimens of the pigs were collected the day before regrouping the pigs (day 3) and suddenly after 30 min regrouping.

2.3 Trial 3

Trial 3 was conducted to investigate some carcass characteristics of pigs. The design was a RBD with repeated measurement. The block and treatment effects were the same factors as trial 1 and 2. Nine finishing barrows (body weights about 90 kg) were randomly chosen for this study. The barrows were reared in a single pen (0.6 × 1.8 m^2) for seven days of adjustment and fed the same normal Trp diet. The dietary treatments (Table 1 and 2) were fed the barrows for four days in the same amount before handling, transportation and slaughter. To achieve minimal handling, the barrows were moved in the cage to a loading area pen by pen. The handling took approximately one minute. After that the pens were loaded on a truck in a common compartment. Barrows were transported by the truck at a speed of about 60 km/h for 2 h, and were then unloaded and slaughtered without rest at a slaughterhouse of Nan's municipality. These handling procedures necessarily induced a relatively high level of stress that could easily produce pale, soft and exudate pork (PSE). Saliva specimens were collected after transportation and before slaughter for detection of salivary cortisol.

2.4 Salivary Cortisol Analysis

Saliva specimen was collected by allowing pigs to chew on hygienic swab cotton until it was fully moistened. The swab samples were placed in a tube and pressed to get about 5 mL of the saliva. Saliva was stored on ice before taking it to the laboratory. Saliva was centrifuged at 3,000 rpm at 4 °C for 5 min and stored at -20 °C for later cortisol analysis. Cortisol was assayed by radioimmunoassay using the Cortisol ^{125}I RIA kit (Coat-a-Count Cortisol TKCO, Diagnostic Products Corp., Apeldoorn, The Netherlands). Gamma rays were counted using the Genesys GENII™ (Laboratory Technology, Inc, IL, United States of America).

2.5 Carcass Evaluation

For measuring carcass quality longissmus muscles (LM) were chosen as the representative sample. The muscles were collected on both sides of the carcass. The pH and temperature of the LM between the 4th and 5th ribs was determined at 45 min postmortem and after 48 h of chilling. The pH meter was a EUTECH pH 6^+, Eutech Inst., Singapore. To determine drip loss, the LM was sharply sliced for 2.5 cm length for 3 pieces per each sample. Then, filter paper was weighed and gently pressed on the caudal cut surface of the LM slice for 10 sec, and subsequently reweighed to determine the absorbed moisture content. A slice of the LM was placed with the cut surface facing down on a metal grid that was placed in a closed plastic container. Drip loss was determined by percentage of weight loss after 24 h of storage at 4 °C.

2.6 Statistical Analysis

Data for each response variable were analyzed by ANOVA using the GLM procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC). The PDIFF option of SAS was used to determine differences between treatment means. Except for the general behaviors of the pig, the data were transformed using a natural logarithm. The transformed data were analyzed following the statistical model given below.

$$Y'_{ijkl} = \mu + O_i + O(P)_{i(j)} + B_k + \tau_l + B\tau_{kl} + \varepsilon_{ijkl}$$

Where, Y'_{ijkl} is the behavior observation in a form of natural logarithm, μ is the overall mean of observation, O_i is the random effect due to the observer i, $O(P)_{i(j)}$ is the nested effect of the observer on the observation day, B_k is the fixed effect due to the breed group of pig k, τ_l is the fixed effect of the dietary treatment l, ε_{ijkl} and is the random residual effect.

The aggressive behaviors of pigs were calculated as the frequency of their expression. This frequency was tested for their dependency on breed group and dietary treatment using χ^2.

3. Results and discussion

3.1 Trial 1

Between periods of study the general behavior most expressed was lying down, with no difference between diets (Table 3). Before startling, pigs fed the supplemental diet L-Trp with twice the control amount (2×-Trp) spent less time eating ($P = 0.04$) and more time lying down ($P = 0.08$). This response agreed many reported studies [11, 16-17]. However, it contradicted a report of no effect of dietary Trp on any behavioral variables in pigs [1, 12, 18]. In addition, dietary Trp can increase brain Trp, and Trp, like other large neutral amino acids (LNAA) [12], can induce large variations in the brain's amino acid and indole concentrations [19].

The period after startling found no difference in behavior among the treatments (Table 3). The response of the pigs to the startling stimulus was a quick alertness and standing up. Gradually, the animals returned to their previous activity over the course of the observation. Likewise, no difference was found for the general behaviors during the post treatment period when all pigs fed on the control diets.

Table 3 Percentage of general behaviors of pigs before and after the startling stimulus during the period fed the different dietary treatments

Behaviors	Dietary Trp Levels			p-value*
	Control	2×-Trp	4×-Trp	
	mean ± SEM † (n = 7) ‡	mean ± SEM (n = 7)	mean ± SEM (n = 7)	
Before startling				
Standing	21.11 ± 0.13	17.39 ± 0.13	18.18 ± 0.13	0.55
Lying	40.70 ± 0.08[b]	51.65 ± 0.08[a]	46.41 ± 0.08[ab]	0.08
Walking	7.30 ± 0.13	5.39 ± 0.13	6.92 ± 0.13	0.28
Eating/drinking	8.61 ± 0.15[a]	4.86 ± 0.15[b]	6.22 ± 0.15[ab]	0.04
Others	22.28 ± 0.13	20.71 ± 0.13	22.27 ± 0.13	0.62
After startling				
Standing	29.27 ± 0.10	26.67 ± 0.10	32.74 ± 0.10	0.32
Lying	28.27 ± 0.10	33.94 ± 0.10	25.44 ± 0.10	0.11
Walking	11.25 ± 0.11	10.13 ± 0.11	12.40 ± 0.11	0.45
Eating/drinking	11.36 ± 0.13	9.09 ± 0.13	9.42 ± 0.13	0.43
Others	19.85 ± 0.13	20.17 ± 0.13	20.00 ± 0.13	0.68

* the p-value greater than 0.05 indicated not significantly different
a, b different superscript within rows indicated a significantly different of treatment mean, the mean of no superscript within rows indicated not significantly different ($p > 0.05$)
† SEM = standard error of mean
‡ Number of pigs per pen

3.2 Trial 2

After regrouping, within one minute, pigs started nosing each other and fighting started. The results showed that different dietary Trp did not affect the frequency expression of the aggressive behavior (Table 4). Related to the preceding is a report that diets containing high Trp levels had no effect on the number of fights [11, 13]. Fighting among pigs consisted mainly of mutual biting, one-way biting and pressing. Aggressive behaviors differed significantly between observation times. During 0-10 min of observing, pigs on every diet fought intensely. For the next period, 10-20 min, the fighting of pigs of the 4×-Trp diet declined by 70% of the fighting of the first 10 min, to only occasional biting. This was higher than a reported 50% decrease in fights reported [13]. During 20-30 min the fighting declined to nearly zero. The lying behavior of pig increased as the mixing time increased (Fig. 1). Enhancing Trp diet can reduce behavioral aggressiveness in growing pigs. The results were likely mediated by the activation of the brain serotonergic system [20], and are related to a report of the neuroendocrine components of stress [11].

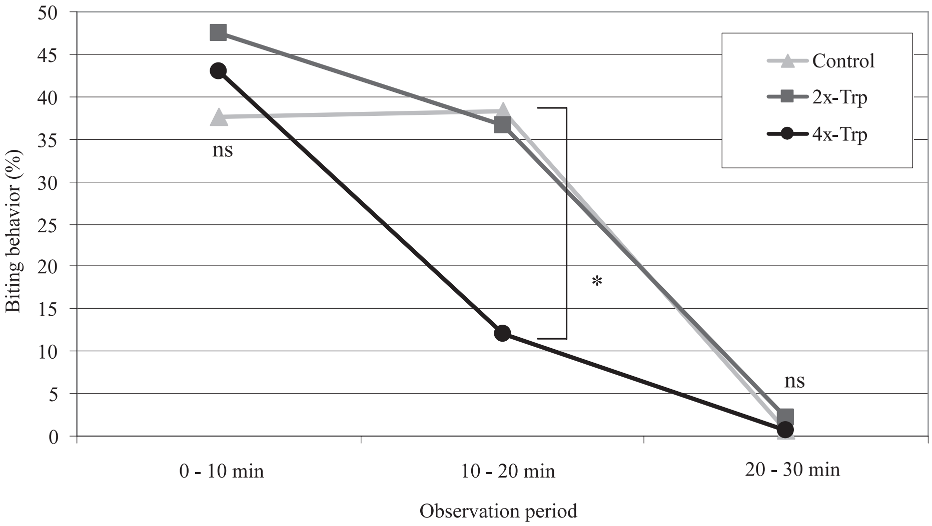
Table 4 The frequency of expression aggressive behaviors of pigs during 30 min regrouping

Behaviors	Dietary Trp Level			p-value*
	Control	2×-Trp	4×-Trp	
	mean ± SEM	mean ± SEM	mean ± SEM	
Parallel pressing	4.67 ± 2.85	5.00 ± 2.85	2.33 ± 2.85	0.78
Inverse parallel pressing	5.33 ± 2.71	3.00 ± 2.71	1.00 ± 2.71	0.57
Parallel pressing-biting	2.00 ± 1.37	6.00 ± 1.37	4.33 ± 1.37	0.23
Mutual bite	22.00 ± 6.27	19.67 ± 6.27	19.00 ± 6.27	0.94
Bite	18.00 ± 5.33	18.00 ± 5.33	14.00 ± 5.33	0.84
Levering	1.00 ± 1.31	2.00 ± 1.31	3.33 ± 1.31	0.51
Head to body knocking	1.00 ± 0.58	0.00 ± 0.58	0.00 ± 0.58	0.44
Head to head knocking	0.67 ± 0.61	0.00 ± 0.61	2.00 ± 0.61	0.17
Nose to head contact	8.00 ± 2.33	10.33 ± 2.33	9.67 ± 2.33	0.78
Nose to body contact	6.33 ± 1.50	4.67 ± 1.50	8.67 ± 1.50	0.28

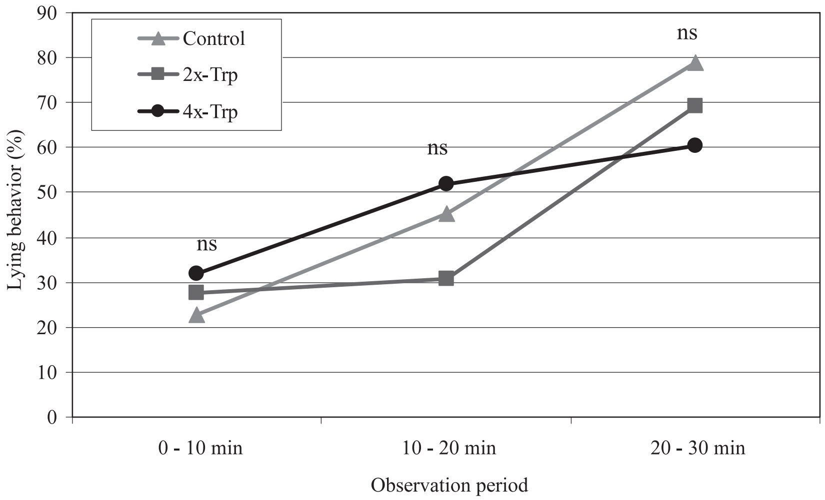
* The p-value greater than 0.05 indicated not significantly different

† SEM = standard error of mean

For the control and the 2×-Trp diets, salivary cortisol during the period before regrouping was significantly ($P < 0.05$) lower than during mixing. For the supplemental 4×-Trp treatment, however, it did not differ for the period before and during mixing (Table 5). This observation indicated that supplemental 4×-Trp could increase pig tolerance to stress and subdue fighting during regrouping. Although 4×-Trp had no effect on the number of fights, it may however contribute to the pig avoiding stressful situations when possible. Short-term high-TRP dietary supplementation may be used to reduce aggression when mixing young pigs [20].



(a)



(b)

Fig. 1 Comparison of the frequency of expression behaviors of pigs during 30 min regrouping (a) biting and (b) lying behaviors

* = significantly different at $p < 0.05$; ns = not significantly different

Table 5 Salivary cortisol levels ((ng/mL)) at the period before and after regrouping

Periods	Dietary Trp Level		
	Control	2×-Trp	4×-Trp
	mean ± SEM [†]	mean ± SEM	mean ± SEM
Before regrouping	7.30 ± 0.81[b]	8.17 ± 0.81[b]	7.71 ± 0.81
After regrouping	10.07 ± 0.81[a]	12.13 ± 0.81[a]	9.76 ± 0.81

[a, b] means in different superscript within columns indicated significantly different ($P \leq 0.05$)
[†] SEM = standard error of mean

3.3 Trial 3

During transportation there were no differences of rectum temperature of the barrows fed different diets (Table 6). The values were in the range of the normal body temperature (38.5 - 39.5°C). However, the salivary cortisol measurement after 2 h of transportation was very high when compared to the normal rest period (before mixing) of trial 2. Cortisol levels were in range of a previously reported study [18]. The handling during transportation can increase cortisol, muscle glycolytic potential, electrical conductivity and drip losses [9, 10]. The 4×-Trp treatment however showed lower cortisol values than the other treatments. This indicated that the animals were highly stressed during this handling, but tryptophan may be a mechanism to reduce the stress response in pigs. A Tryptophan supplemented diet seemed to influence the action of neurotransmitters, therefore offering a practical way of reducing stress response in swine [1, 18, 20].

Table 6 Comparison the values of body temperature and cortisol of pigs after handling by transportation and some carcass characteristics after slaughtering

Item	Dietary Trp Level		
	Control	2×-Trp	4×-Trp
	mean ± SEM	mean ± SEM	mean ± SEM
Rectum temperature (°C)	38.53 ± 1.01	38.90 ± 0.89	38.73 ± 1.04
Salivary cortisol (ng/mL)	14.61 ± 0.64[a]	15.63 ± 0.64[a]	11.62 ± 0.64[b]
Temperature 45 min postmortem (°C)	35.13 ± 0.21[a]	34.39 ± 0.21[b]	34.68 ± 0.21[b]
Temperature 48 h chilling (°C)	9.36 ± 0.18	9.50 ± 0.18	9.11 ± 0.18
pH 45 min postmortem	5.49 ± 0.11	5.79 ± 0.11	5.53 ± 0.11
pH 48 h chilling	5.47 ± 0.06	5.46 ± 0.06	5.41 ± 0.06
Drip loss percentage (%)	8.87 ± 0.46[a]	3.83 ± 0.46[c]	7.61 ± 0.46[b]

[a, b, c] the treatment mean in different superscript within rows indicated significantly different ($P \leq 0.05$), and the treatment mean of no superscript within rows indicated not significantly different ($P > 0.05$)
[†] SEM = standard error of mean

It has been reported that supplementation with 6g of Trp/kg of feed for 5 days was able to increase the plasma Trp concentration [16]. However, if additional suboptimum Trp for growth (0.12%) is supplied, it can depress serotonin concentration that relates to a more critical serotonergic activity when hypothalamic serotonin concentration falls below a threshold level [14].

Of significance was the temperature difference of the longissmus muscle (LM) 45 min postmortem of the animals. Pigs fed the control diet had the highest carcass temperature ($P < 0.05$). No significant differences were found for the 48 h temperature, 45 min pH postmortem and 48 h pH for pigs fed different dietary Trp. However, the LM pH was lower than in a previously reported study using an additional 5 g of Trp per kilogram of a 14% crude protein diet in pigs [1]. Peeters and others [17] found no differences among treatments of diets supplemented with magnesium, tryptophan, vitamin C, vitamin E, and herbs on pH and temperature in the LM 45 min and 48 h postmortem.

Drip loss percentage was found to be high in pigs fed the control diet (Table 5) and differed significantly from other treatments ($P < 0.05$). This verifies that pigs fed the control Trp diet were highly stressed during transportation and slaughter. The drip loss of the low Trp diet may be evidence of pork revealing pale, soft and exudative (PSE) meat. In addition, high stress during transportation can decrease the color of redness (a*) and yellowness (b*) values [10], and therefore produce an undesirable discoloration. The supplementation 2×-Trp diet, however, had the lowest drip loss and evidenced dark, firm and dry (DFD) meat. These may be the effect of the high pH at 45 min postmortem. The proteins in high pH meat can increase the ability to hold water and produce less drip loss. This was one of the effects of preslaughter stress. Moreover, high preslaughter stress leads to impaired pork quality, with high muscle energy levels aggravating the negative effects of preslaughter stress [9] and was the major factor responsible for reductions in pork quality [9-10].

4. Conclusion

The preceding observations answer the question posed at the beginning of the work. As a reassurance to the Thai pork industry, this research concluded that L-Trp could be supplemented for short-term use. The supplementation in the diet should be for up to 4 times the amount needed for growth. The regimen reduced the stress in pigs during regrouping and transportation, and may even improve the pork quality. It also answers the concern consumers may have of the pig's welfare prior to slaughter.

5. Acknowledgements

The authors gratefully thank the National Research Council of Thailand (NRCT) for the funding. The department of animal science and fisheries, Rajamangala University of Technology Lanna, Nan campus, is acknowledged for providing the facility to support this research. We thank Dr. Rainer Zawadzki, RMUTL, for editing the manuscript. We also would like to acknowledge "Hands-on research and development project" of Rajamangala University of Technology Lanna.

6. References

1. Adeola, O. and Ball, R.O., 1992, "Hypothalamic neurotransmitter concentrations and meat quality in stressed pigs offered excess dietary tryptophan and tyrosine", *Journal of Animal Science*, Vol. 70, pp. 1888-1894.

2. Pethick, D.W., Warner, R.D., D'Souza, D.N.,

and Dunshea, F.D., 1997, *Nutritional Manipulation of Meat Quality*, in P.D. Cranwell (Ed.) Manipulating Pig Production VI, Australia Pig Science Association, Canberra.

3. Huether, G., Kochen, W., Simat, T.J., and Steinhart, H., 1999, *Tryptophan, Serotonin, and Melatonin: Basic Aspects and Applications*, Kluwer Acad Plenum Pub, New York.

4. Sève, B., 1999, *Physiological Roles of Tryptophan in Pig Nutrition*, in G. Huether (Ed.), Tryptophan, Serotonin, and Melatonin: Basic Aspects and Applications. Kluwer Acad Plenum Pub, New York.

5. Ekkel, E.D., Savenije, B., Schouten, W.G.P., Wiegant, V.M., and Tielen, M.J.M., 1997, "The effects of mixing on behavior and circadian parameters of salivary cortisol in pigs", *Physiology Behavior*, Vol. 62, pp. 181–184.

6. Pluske, J.R., Hampson, D.J., and Williams, I.H., 1997, "Factors influencing the structure and function of the small intestine in the weaned pig: A review", *Livestock Production Science*, Vol. 51, pp. 215-236.

7. Jensen, P. and Yngvesson, J., 1998, "Aggression between unacquainted pigs sequential assessment and effects of familiarity and weight", *Applied Animal Behaviour Science*, Vol. 58, pp. 49-61.

8. Ruis, M.A.W., Brake, J.H., van de Burgwal, J.A., Jong, I.C., Blokhuis, H.J., and Koolhaas, J.M., 2000, "Personalities in female domesticated pigs: behavioral and physiological indications", *Applied Animal Behaviour Science*, Vol. 66, pp. 31-47.

9. Hambrecht, E., Eissen, J.J., Nooijen, R.I.J., Ducro, B.J., Smits, C. H.M., den Hartog L.A., and Verstegen, M.W.A., 2004, "Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants", *Journal of Animal Science*, Vol. 82, pp. 1401-1409.

10. Hambrecht, E., Eissen, J.J., Newman, D.J., Smits, C.H.M., den Hartog, L.A., and Verstegen, M.W.A., 2005, "Negative effects of stress immediately before slaughter on pork quality are aggravated by suboptimal transport and lairage conditions", *Journal of Animal Science*, Vol. 83, pp. 440-448.

11. Koopmans, S.J., Guzik, A.C., Meulen, J., Dekker, R., Kogut, J., Kerr, B.J., and Southern, L.L., 2006, "Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weaning piglets", *Journal of Animal Science*, Vol. 84, pp. 963-971.

12. Sève, B., Meunier-Salaun, M.C., Monnier, M., Colle´ux, Y., and Henry, Y., 1991, "Impact of dietary tryptophan and behavioral type on growth performance and plasma amino acids of young pigs", *Journal of Animal Science*, Vol. 69, pp. 3679-3688.

13. Li, Y.Z., Kerr, B.J., Kidd, M.T., and Gontou, H.W., 2006, "Use of supplementary tryptophan to modify the behavior of pig", *Journal of Animal Science*, Vol. 84, pp. 212-220.

14. Henry, Y., Sève, B., Mounier, A., and Ganier, P., 1996, "Growth performance and brain neurotransmitters in pigs as affected by tryptophan, protein, and sex", *Journal of Animal Science*, Vol. 74, pp. 2700-2710.

15. Ruis, M.A.W., 2001, *Social Stress as a Source of Reduced Welfare in Pigs*, Ph.D. Dissertation, the University of Groningen, the Netherlands.

16. Peeters, E., Neyt, A., Beckers, F., De Smet, S., Aubert, A.E. and Geers, R., 2005, "Influence of supplemental magnesium, tryptophan, vitamin C, and vitamin E on stress responses of pigs to vibration", *Journal of Animal Science*, Vol. 83, pp. 1568-1580.

17. Peeters, E., Driessen, B., and Geers, R., 2006, "Influence of supplemental magnesium, tryptophan, vitamin C, vitamin E, and herbs on stress responses and pork quality", *Journal of Animal Science*, Vol. 84, pp. 1827-1838.

18. Adeola, O., Ball, R.O., House, J.D. and O'Brien, P.J., 1993, "Regional brain neurotransmitter concentrations in stress-susceptible pigs", *Journal of Animal Science*, Vol. 71, pp. 968-974.

19. Meunier-Salaün, M.C., Monnier, M., Colleaux, Y., Sève, B., and Henry, Y., 1991, "Impact of dietary tryptophan and behavioral type on behavior, plasma cortisol, and brain metabolites of young pigs", *Journal of Animal Science*, Vol. 69, pp. 3689-3698.

20. Poletto, R., Meisel, Richert, Cheng, H-W., and Marchant-Forde, 2010, "Aggression in replacement grower and finisher gilts fed a short-term high tryptophan diet and the effect of long-term human-animal interaction", *Applied Animal Behaviour Science*, Vol. 122, pp. 98-110.