# The effect of dietary I-tryptophan on productive performance and behavior of weaned piglets

# ŽIVKOVIĆ VLADIMIR<sup>1</sup>\*, STANKOVIĆ BRANISLAV<sup>2</sup>, HRISTOV SLAVČA<sup>2</sup>, DELIĆ NIKOLA<sup>1</sup>, NIKŠIĆ DRAGAN<sup>1</sup>, SAMOLOVAC LJILJANA<sup>1</sup>, PETRIČEVIĆ MAJA<sup>1</sup>

<sup>1</sup> Institute for Animal Husbandry, Autoput 16, 11080 Belgarde, Serbia

<sup>2</sup> University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia

## SUMMARY

This study was carried to determine if dietary tryptophan can be beneficial for piglets in period of weaning. Trial was conducted on 84 individuals (Landrace×Yorkshire) female and castrated male piglets, at 30 days of age, and of 9.78±0.42 kg. Test subjects were penned into four groups and allocated to four different diets. This was done opposite to standard farm procedure; all piglets penned in same group came from different litters. Animals were fed *ad libitum* for six days. Piglets in trial groups were fed with basically same mixture with different levels of digestible L-tryptophan (0.1; 0.2; 0.3%). Productive performance (FCR; ADG; ADFI) was calculated and behavior characteristics (postures, mounting, abnormal and aggressive behavior) were observed using CCTV cameras. For the purpose of production performance analysis one way ANOVA was used while the Tukey test served to determine the statistical significance of the differences between individual means values. Considering that there is no normal distribution for behavioral parameters, we used non parametric Kruskal-Wallis test with multiple comparisons of mean rank between groups. Productive results showed that control group had significantly better results for average daily gain compared to all trial groups (p<0.05), other productive parameters didn't show any significant difference. On the other hand, statistically significant results occurred for two behavioral characteristics. Fighting differed significantly (p<0.05), during first day of weaning between control and trial groups, intensive ear biting occurred at day two and three after weaning, and different significantly on day three (p<0.05). According to our results tryptophan had no positive effects on productive performance (feed intake, daily weight gain and feed conversion), but had some positive effects on reducing aggressive behavior. Conclusion could be that implementing small doses of tryptophan on weaning can be beneficial to reducing stress and behavioral anomalies of piglets. Further more extensive studies should be carried to verify these results.

## **KEY WORDS**

Rearing, stress, nutrition, aggressive behavior.

## INTRODUCTION

In commercial swine production, weaning is probably one of the most stressful periods in the life of a pig. Intensive swine production has led to decreasment of weaning age. For better utilization of housing facilities piglets were usually reared at an early stage of life<sup>1,2</sup>. Today, most piglets on commercial farms are separated from their mothers at the age of 3 to 4 weeks<sup>3</sup>. Piglets experience certain change at a time when usually they are not supposed to face them. Those changes include: separation from the sow, change of environment, mixing with piglets from different litters and change from a liquid based diets to a solid ones. Weaning exposes piglets to different conditions that affect their welfare, which in turn causes major stress. Tryptophan is known to perform several physiological functions. When tryptophan is supplemented in higher amounts than the requirements, it can be used as a therapeutic supplement. Tryptophan has been shown to affect brain and nervous system function through interference with serotonergic neurotransmission<sup>4</sup>. Tryptophan is widely regarded as fourth or fifth limiting amino acid (AA) in maize based diets. In some studies tryptophan has been associated with the control of stress, immune response and health maintenance<sup>5</sup>. Tryptophan serves as precursor for serotonin synthesis, and tryptophan-induced serotonergic activity in the brain has been associated with the regulation of some behavioral and physiological processes such as change of mood, control of aggression, sensitivity to stress, sleep patterns, and feed intake<sup>6.7</sup>.

Behavioral observations had already been made in numerous studies, mostly dealing with the dominance in the relationships, which are often connected with other behavioral and physiological characteristics of the animals<sup>8</sup>. Rearing environment can influence the development of some behavior, which can also influence behavioral response to stressful situations in later stages of life<sup>2</sup>.

We hypothesized that an increase in tryptophan concentration in the diet above the assumed normal requirement value would reduce behavioral and physiological stress at weaning and that it will positively affect productive performance of piglets.

## MATERIALS AND METHODS

#### Diet and housing

Trial was conducted on experimental farm of Institute for animal husbandry, Belgrade, Serbia. Crossbred (Landrace×Yorkshire) female and castrated male piglets, at 30 days of age and

Corresponding Author: Živković Vladimir (vladimirzivkovic\_87@yahoo.com).

bodyweight of 9.78±0.42 kg, were used in trial. Piglets from seven litters of ten pigs each were penned in four groups of seven (4:3; male to female ratio) and allocated to four different diets. This was done opposite to standard farm procedure; all piglets penned in same group came from different litters. Trial comprised 3 replications with total of 84 piglets. Pen was rectangle shape, three square meters in size, with seven separate feeding places. Every pen is equipped with one nipple drinker. Pens are cage based with combined floors (concrete and plastic). Ventilation, temperature and relative humidity were constant during the trial.

Animals were fed *ad libitum* for six days. All animals were fed morning before weaning with trial mixtures, so that tryptophan can take function before weaning. The feed was pre-weighed at start of trial in daily portions for each pen according to the expected feed consumption.

The pigs had access to the feed at all times through manual feeders placed in the front of each pen.

Average daily feed consumption (ADFI) was calculated by subtracting unconsumed feed at the end of trial from the preweighed amount and splited by the days. Body mass were weighted at start and at the end of trial. Piglets were weighed at the beginning and at the end of the experiment and the Average daily gain (ADG) were calculated with the following equation:

$$ADG = \frac{Final weight - Initial weight}{Duration of the experiment (days)}$$

Feed conversion (FCR) was also calculated:

$$FCR = \frac{\text{Daily feed intake}}{\text{Daily weight gain}}$$

A basal mixture (Table 1) was formulated to contain sufficient amounts of all essential amino acids. The piglets in trial groups were fed with basically same mixture with different levels of digestible L-tryptophan (0.1; 0.2; 0.3%, respectively).

Table 1 -	Composition	of diets for weaned	piglets in the trial.
-----------	-------------	---------------------	-----------------------

Treatment	С	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Ingredients, g/kg				
Maize	578.00	577.90	577.80	577.70
Soybean cake	240.10	240.10	240.10	240.10
Soybean meal	100.00	100.00	100.00	100.00
Calcium carbonate	15.00	15.00	15.00	15.00
Sodium chloride	3.70	3.75	3.75	3.75
Monocalcium phosphate	10.10	10.10	10.10	10.10
L-Lysine	1.10	1.10	1.10	1.10
L-Tryptophan	-	1.00	2.00	3.00
Minazel*	2.00	2.00	2.00	2.00
Mineral-vitamin premix**	50.00	50.00	50.00	50.00

\* Natural mycotoxin adsorbent.

\*\* Added per kg diet: 15,000 IU Vitamin A, 1500 IU Vitamin D3, 40 IU Vitamin E, 1.0 mg Vitamin K3, 2.0 mg Vitamin B1, 4 mg Vitamin B2, 10 mg d-Pantothenic acid, 18 mg Niacin, 70 mg Biotin, 18 mg Vitamin C, 0.03 mg Vitamin B12, 4 mg Vitamin B6, 170 mg Fe: Fe(II) sulphate, 4 mg Cu: Cu(II) sulphate, 16 mg Zn: Zn(II) oxide, 50 mg Mn: Mn(II) oxide, 0.304mg KI, 0.3 mg Se: Se-selenite.

Table 2 - Ethogram of behaviors observed of	during continuous observations of all	pigs in the experiment after mixing <sup>10</sup> .

Behavior	Description
<i>Posture</i> Lying Sitting Standing	Pig lying with eyes open or with eyes closed and without movement. Dog-sitting position. Pig standing on all four feet.
<i>Aggressive</i> Fights Headknocks	Mutual pushing, ramming or pushing the opponent with the head, with or without biting. Lifting the opponent by pushing the head under its body <sup>11</sup> . Knocking heads between two pigs. Three or more knocks is considered a fight <sup>12</sup> .
<i>Mounting</i> Mounts	Placing feet on the back of another pig with or without pelvic movement <sup>13</sup> .
Abnormal Tail directed Ear directed Flank directed	Tail positioned in the mouth of another pig. Ranges from tail being gently manipulated to tail being chewed or bitten <sup>14</sup> . Ear positioned in the mouth of another pig: ranges from ear being gently manipulated to being chewed or bitten <sup>14</sup> . Biting directed towards the flank of another pig.

Table 3	<ul> <li>Statistical</li> </ul>	indicators	(mean :	E SE) for	production	performance of	piglets.
---------	---------------------------------	------------	---------	-----------	------------	----------------	----------

Treatments	С	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
ADFI, g/d	324.29±0.05	319.68±0.06	303.97±0.07	294.21±0.09
ADG, g/d	154.60±0.02 <sup>a</sup>	132.85±0.03 <sup>b</sup>	132.22±0.03b	115.47±0.01 <sup>b</sup>
FCR, g/g	2.09±0.15	2.40±0.22	2.29±0.06	2.54±0.17

SEM, Standard error of the means; ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion rate; <sup>a,b</sup>, in a row, the least squares means with a different superscript differ significantly (p<0.05).

## **Behavior**

Observation of test subject was done by using four CCTV cameras which recorded from 6:00h to 20:00h for 6 days post weaning. Cameras were placed on the ceiling at three meters height, so it can provide us top view of the pen. All cameras equipped with motion detection sensor and night recording. Color images were captured with a frame rate of 24 frames per second and a resolution of  $1280 \times 720$  pixels. All observed behaviors are classified in table 2. For the analysis of the collected date we used Behavioral Observation Research Interactive Software<sup>9</sup>.

#### Statistical analyses

All statistical analyses were performed using R-project software<sup>15</sup>. For the purpose of production performance analysis one way ANOVA was used while the Tukey test served to determine the statistical significance of the differences between individual means values. Considering that there is no normal distribution for behavioral parameters, we used non parametric Kruskal-Wallis test with multiple comparisons of mean rank between groups.

#### RESULTS

#### Production performance

Results (Table 3) have shown that control group had better production results for both ADG and FCR compared to the trial groups. Only statistical significance occurred in ADG between control group and the rest.

#### Behavior

**Postures.** Treatments had no significant effect on the postures of pigs in all groups (P>0.05).

**Aggressive behavior.** There were significantly more aggressive behaviors in control group compared to trial (Figure 1) during first 24h post weaning (P<0.05). Fighting time in pens differed from 1 to 256 seconds.

**Mounting behavior.** All mounts occurred in the first three days after mixing. Mounts lasted from 3 to 12 seconds. There were no statistical significance between treatments (P>0.05).

Abnormal behavior. On the third day there were statistical difference between control and trial groups (P<0.05), and also there were very big difference between days. First 24h post weaning

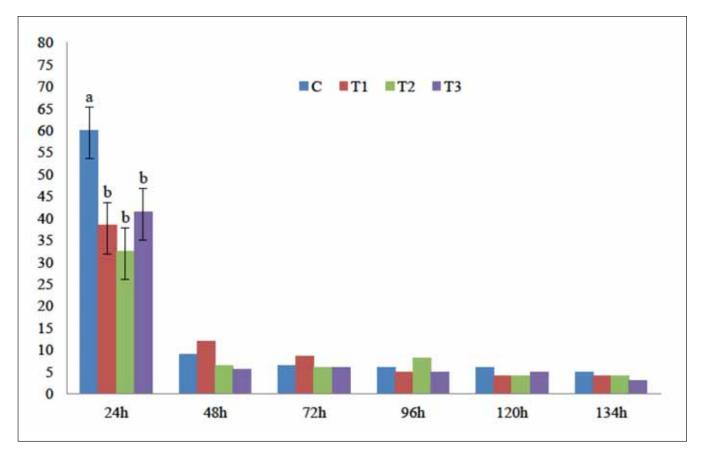


Figure 1 - Number of fights for the whole trial period, Different letters indicate significant differences (P<0.05).

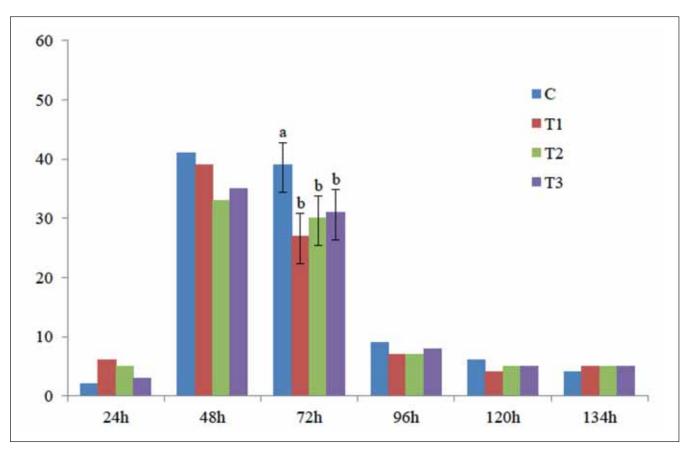


Figure 2 - Number of cases of abnormal behaviors for the whole trial period; Different letters indicate significant differences (P < 0.05).

there were only few cases of abnormal behavior. Than in next couple of day's number of cases multiplied several times (Figure 2). On the fourth day again number of cases dropped.

## DISCUSSION

Pigs are very adaptable to their environment, but nutritional status may affect how they respond to environmental stimulus. Apart from being a one of the major amino acids for protein synthesis, brain serotonin content is increased as the result of increased dietary tryptophan<sup>16</sup>, and also plays a key role in appetite regulation.

Our result has shown that dietary l-tryptophan had no positive effect on growth performance of the piglets. Control group had best results for ADG and FCR. Some researchers come to same conclusion as us, that dietary tryptophan had no effect on daily gain<sup>17, 18</sup>; while others concluded tryptophan in small amounts could improve ADG and FCR<sup>19, 20</sup>.

Martínez-Trejo et al.<sup>21</sup> also reported that tryptophan supplementation of piglets positively affected some categories related with their behavior, however, it had no effect on their productive performance (feed intake, daily weight gain and feed conversion), which is pretty similar to our results. This suggests that when giving tryptophan above the requirements, feed intake does not decrease. Those results also agrees with the one reported by Li et al.<sup>7</sup>. Janczak et al.<sup>22</sup>, observed a tendency to decrease aggressive behavior among animals when supplementing tryptophan in the drinking water of mice. Meunier-Salaün et al.<sup>16</sup> reported that dietary tryptophan levels induced minor changes in behavioral responses. Some researchers concluded that adding dietary tryptophan induces lethargic behavior<sup>23</sup>. Peeters et al.<sup>24</sup> reported that pigs provided with tryptophan in their drinking water spent more time lying during simulated transport than control pigs, but no other differences were observed.

When tryptophan is supplemented, some nutritional factors can influence the passage of tryptophan across the blood-brain barrier to convert it into serotonin. Level of the Large Neutral Amino Acids (LNAA; leucine, isoleucine, methionine, valine, phenylalanine and tyrosine) in diets, are competing with tryptophan for carrier proteins across the cell membrane; and also level of carbohydrates in the diet where high glycemic index can increase insulin which results removal of selectively the LNAA from plasma with less effect on tryptophan<sup>25</sup> and also the concentration of fatty acids, which compete with tryptophan for binding to albumin<sup>26</sup>.

## CONCLUSION

According to our results tryptophan had no positive effects on productive performance (feed intake, daily weight gain and feed conversion), but had some positive effects on reducing aggressive behavior. Conclusion could be that implementing small doses of tryptophan on weaning can be beneficial to reducing stress and behavioral anomalies of piglets. Further detailed studies must be conducted to verify these results.

## ACKNOWLEDGEMENTS

Research was financed by the Ministry of Education, Science and Technological Development of Republic of Serbia No. 451-03-68/2020-14.

#### References

- 1. Benson G.J., Rollin B.E. (2004). The well-being of farm animals: challenges and solutions. Blackwell Publishing, Ames, IA: USA.
- Li Y., Wang L. (2011). Effects of previous housing system on agonistic behaviors of growing pigs at mixing. Appl. Anim. Behav. Sci., 132: 20-26.
- Fels M., Hoy S., Hartung J. (2012). Influence of origin litter on social rank, agonistic behaviour and growth performance of piglets after weaning. Appl. Anim. Behav. Sci., 139: 225-232.
- Huether G., Kochen W., Simat T.J., Steinhart H. (1999). Tryptophan, serotonin, and melatonin: Basic aspects and applications. Kluwer Academic/Plenum Publ., New York.
- Le Floc'h N., Sève B. (2007). Biological roles of tryptophan and its metabolism: potential implications for pig feeding. Livest, 112: 23-32.
- Markus C.R., Olivier B., Panhuysen G.E.M., van der Gugten J., Alles M.S., Tuiten A., Westenberg H.G., Fekkes D., Koppeschaar H.F., de Haan E.E. (2000). The bovine protein alpha-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. Am. J. Clin. Nutr., 71: 1536-1544.
- Li Y.Z., Kerr B.J., Kidd M.T., Gonyou H.W. (2006). Use of supplementary tryptophan to modify the behavior of pigs. J. Anim. Sci., 84: 212-220.
- 8. Langbein J., Puppe B. (2004). Analysing dominance relationships by sociometric methods - a plea for amore standardised and precise approach in farm animals. Appl. Anim. Behav. Sci., 87: 293-315.
- 9. Friard O.P., Gamba M. (2016). Behavioral Observation Research Interactive Software (BORIS). Università di Torino.
- van Staaveren N., Lemos Teixeira D., Hanlon A., Ann Boyle L. (2015). The Effect of Mixing Entire Male Pigs Prior to Transport to Slaughter on Behaviour, Welfare and Carcass Lesions. PLoS One., 10(4).
- Stewart C.L., O'Connell N.E., Boyle L. (2008). Influence of access to straw provided in racks on the welfare of sows in large dynamic groups. Appl. Anim. Behav. Sci., 112: 235-247.
- Keeling L.J., Gonyou H.W. (2001). Social Behavior in Farm Animals. CAB International, Wallingford, UK, 147-176.
- Fàbrega E., Puigvert X., Soler J., Tibau J., Dalmau A. (2013). Effect of on farm mixing and slaughter strategy on behaviour, welfare and productivity in Duroc finished entire male pigs. Appl. Anim. Behav. Sci., 143(1): 31-39.
- O'Connell N.E., Beattie V.E., Watt D. (2005). Influence of regrouping strategy on performance, behaviour and carcass parameters in pigs. Livestock Production Science, 97: 107-115.
- 15. R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

- Meunier-Salaün M.C., Monnier M., Colléaux Y., Seve B., Henry Y. (1991). Impact of dietary tryptophan and behavioral type on behavior, plasma cortisol and brain metabolites of young pigs. J. Anim. Sci., 69: 3689-3698.
- Susenbeth A., Lucanus U. (2005). The effect of tryptophan supplementation of diets of restricted and unrestricted-fed young pigs. J. Anim. Physiol. Anim. Nutr. (Berl.), 89 (9-10): 331-336.
- Gomes L.M., ade Mello Miassi G., dos Santos L.S., Dib Saleh M.A., Sartori J.R., Panhoza Tse M.L., Berto D.A. (2018). Impact of two light programs and two levels of dietary tryptophan for weanling piglets. Livestock Science, 216: 191-196.
- Trevisi P, Melchior D, Mazzoni M, Casini L, De Filippi S., Minieri L., Lalatta-Costerbosa G., Bosi P. (2009). A tryptophan enriched diet improves feed intake and growth performance of susceptible weanling pigs orally challenged with E.coli K88. J. Anim. Sci., 87: 148-156.
- Capozzalo M.M., Kim J.C., Htoo J.K., de Lange C.F.M., Mullan B.P., Resink J., Hansen C., Stumbles P., Hampson D., Ferguson N., Pluske J. (2020) Estimating the standardized ileal digestible tryptophan requirement of pigs kept under commercial conditions in the immediate post-weaning period. Animal Feed Science and Technology, 259: 114342.
- Martinez-Trejo G., Ortega-Cerrilla M.E., Rodarte-Covarrubias L.F., Herrera-Haro J.G., Figueroa-Velasco J.L. Galindo-Maldonado F., Sanchez-Martinez O., Lara-Bueno A. (2009) Aggressiveness and Productive Performance of Piglets Supplemented with Tryptophan. Journal of Animal and Veterinary Advances, 8: 608-611.
- Janczak A.M., Bakken M., Braastad B.O. (2001). A cautionary note regarding the use of nutritional L-tryptophan to alter aversion-related behavior in mice. Applied Anim. Behav. Sci., 72: 365-373.
- Koopmans S.J., Guzik A.C., van der Meulen J., Dekker R., Kogut J., Kerr B.J., Southern L.L. (2006). Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets. J. Anim. Sci., 84: 963-971.
- Peeters E., Driessen B., Steegmans R., Henot D., Geers R. (2004). Effect of supplemental tryptophan, vitamin E, and a herbal product on responses by pigs to vibration. J. Anim. Sci., 82: 2410-2420.
- 25. Clark J., Mills D.S. (1997). Design Considerations for the Evaluation of Tryptophan Supplementation in the Modification of Equine Behavior. In: Mills, D.S., S.E. Heath and L.J. Harrington (Eds.). Page 164-173 in Proc. of the First International Conference on Veterinary Behavioural Medicine. Universities Federation for Animal Welfare, Potters Bar, April 1-2, Birmingham, UK.
- Grimmett A., Sillence M.N. (2005). Calmatives for the excitable horse: A review of L-tryptophan. Vet. J., 170: 24-32.