Journal homepage: http://www.sasas.co.za/journals

South African Journal of Animal Science 2018, 48 (No. 3)

Effects of vitamin B6 and tryptophan on pork quality and amount of lean meat in gilts of 70–100 kg bodyweight

L.D. Castilha[#], L.A.C. Esteves, L.P. Bonagurio, L.M.D. Huepa, M.R. Fachinello, M.S.S. Pozza, A.C. Furlan & P.C. Pozza

Department of Animal Science, Universidade Estadual de Maringá, Colombo Avenue, 1590, Zip Code 87.020-900, Maringá, Paraná, Brazil.

(Received 23 January 2018; Accepted 14 February 2018; First published online 16 June 2018)

Abstract

Supplementary tryptophan in pig diets has shown improvement in carcass traits and pork quality by reducing the animals' response to stress at slaughter. Vitamin B6 could enhance this response since it acts as an enzymatic cofactor of many tryptophan pathways. The present experiment was designed to evaluate dietary vitamin B6 supplementation and tryptophan levels on carcass traits, organ weights, abdominal fat, and pork quality of 70–100 kg gilts. Sixty-four crossbred gilts (initial bodyweight (BW) 70.52 ± 2.95 kg) were distributed in a 2 x 4 factorial scheme, consisting of two supplementary vitamin B6 levels (1 and 5 mg kg^{-1}) and four dietary standardized ileal digestible (SID) tryptophan (Trp) levels (0.140%, 0.167%, 0.194%, and 0.221%). No significant interactions between the dietary SID Trp levels and B6 supplementation were observed on these variables. Vitamin B6 supplementation (5 mg kg^{-1}) showed a minor reduction in meat pH measured 24 hours after slaughter and resulted in a ham yield higher than B6 basal level (1 mg kg^{-1}). The lean meat yield increased linearly as the SID Trp levels increased in the diet. These findings suggested that vitamin B6 supplementation enhanced the pork quality and the increasing levels of SID tryptophan enhanced the lean meat yield of 70–100 kg gilts.

Keywords: Carcass yield, meat colour, organ weight
[#] Corresponding author: leandrocastilha@hotmail.com

Introduction

Tryptophan (Trp) is generally considered the second or third limiting amino acid in typical corn-soybean meal diets fed to finishing pigs (Lewis & Southern 2000; Cromwell, 2004). In this sense, because the reduction in dietary crude protein is a desirable goal for the pig industry, for economic and environmental reasons (Kerr *et al*., 1995), supplementing industrial amino acids in swine diets becomes an important practice. Furthermore, low crude protein diets with high Trp levels could enhance the serotonin (5-hydroxytryptamine (5-HT)) synthesis (Le Floc'h & Sève, 2007; Le Floc'h *et al*., 2010), which is a neurotransmitter that regulates feed intake and day-night rhythm, and affects pig behaviour (Le Floc'h *et al*., 2011; Li *et al*., 2011; Shen *et al*., 2012; Poletto *et al*., 2014).

Trp deficiency can induce a growth performance depression (Henry *et al*., 1992; Li *et al*., 2006; Shen *et al*., 2012) and decrease 5-HT concentration in the brain (Le Floc'h & Sève, 2007; Le *et al*., 2010). According to Haese *et al.* (2006), beyond improving feed intake, performance and behaviour, supplementary Trp has been added to finishing pigs' diets to improve carcass traits and meat quality by reducing the animals' response to stress at slaughter and therefore the PSE meat type (pale, soft and exudative).

However, the enzymes acting on the metabolization of Trp into serotonin, as well as melatonin, kynurenine, quinurenic acid, xanturenic acid, quinolinic acid, and niacin, use vitamin B6 (B6) as a cofactor, catalysing many biochemical pathways (Matte *et al*., 2011). The daily requirement of B6 that is considered is 1 mg kg^{-1} (NRC, 2012), and is commonly achieved by adding vitamin premixes to pig diets, but recent research results (Matte *et al*., 2005; Zhang *et al*., 2009; Matte *et al*., 2011) suggested B6 requirements five times greater than this.

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v48i3.14

Few studies simultaneously evaluated Trp and B6 in pig diets and their effects on performance, carcass traits and pork quality. Because gilts are more sensitive than barrows to lack of Trp (Henry *et al*., 1996), a shortage from an imbalance between Trp and long-chain neutral amino acids (LNAA), such as valine, isoleucine, leucine, phenylalanine, and tyrosine, could be reduced when supplying Trp and B6 in the diets. It could occur because of reduced competition between LNAA and Trp for the same binding site on the blood brain barrier (Le Floc'h & Sève, 2007).

In this sense, the objective of this study was to evaluate dietary tryptophan levels and vitamin B6 supplementation on carcass traits, organ weights, abdominal fat and pork quality of 70–100 kg gilts.

Material and Methods

An experiment was carried out at the experimental farm of Maringá State University, Brazil. The experiment followed the procedures approved by Maringá State University Animal Care and Use Committee (Protocol number 036/2013).

Sixty-four crossbred gilts (Piétrain x Landrace x Large White) were used, averaging 70.52 ± 2.95 kg initial BW and 99.17 ± 5.77 kg final BW. The pigs were housed in an open-sided finishing barn (2.20 × 1.00 m), with a solid cement floor and shallow pool area (0.10 m deep), and equipped with semi-automatic individual feeders and nipple drinkers (free access to feed and drinking water) in a masonry building. During the experiment, the temperature and relative humidity inside the finishing barn were registered daily (in average 23.4 ± 6.2 ºC and 68.2 ± 13.9%, respectively). Initial BW did not differ (P >0.05) between groups. The animals were allotted to treatments in a randomized block design with eight replicates, each consisting of one gilt.

A 2 x 4 factorial arrangement was used, consisting of two B6 levels (1 and 5 mg kg^{-1}) and four SID Trp levels (0.140%, 0.167%, 0.194%, and 0.221%). The basal B6 level (1 mg kg^{-1}) was proposed by Rostagno *et al.* (2011) and the supplementary one exceeds four times the requirement, based on recent literature (Matte *et al.*, 2005; Zhang *et al*., 2009; Matte *et al*., 2011). Diets were based on corn and soybean meal (Table 1) and were formulated according to the requirements proposed by Rostagno *et al.* (2011), except for Trp and crude protein concentrations. All diets were supplemented with L-lysine, DL-methionine, L-threonine, and L-valine to provide the requirements proposed by Rostagno *et al.* (2011). L-Trp and B6 (pyridoxine 99%) were added to the basal diet (0.140% SID Trp and 1 mg kg^{-1} B6) to ensure the levels of these nutrients. Glutamic acid was used in the experimental diets to provide the same level of nitrogen in all diets.

At the end of the experiment the pigs were fasted for 24 hours and weighed (slaughter weight) before being slaughtered in the abattoir of the experimental farm. Pigs were submitted to electrical stunning (200 watts) and exsanguination. After that, the animals were shaved and gutted.

Carcasses were halved down the midline. Hot carcass yield was the weight sum of the two carcass halves measured at the slaughter house prior to the carcass going into the chiller and expressed as a percentage of hot carcass weight. The carcasses were chilled (1–2 ºC) for 24 hours and then weighed. Cold carcass yield was the weight sum of the two carcass halves measured after chilling and expressed as a percentage of hot carcass weight.

Carcasses were submitted to quantitative evaluation following the Brazilian method of swine carcass classification (Bridi & Silva, 2009) and the American method (NPPC 2000). After chilling, the left ham of each carcass was removed and weighed (ham weight), and the ham weight was expressed as a percentage of cold carcass weight to determine the ham yield.

At slaughter, the spleen, liver, and kidneys were weighed to obtain the relative organ weight, based on the hot carcass weight. Abdominal fat was also removed and weighed to obtain its relative weight, based on the cold carcass weight.

An optical system (Hennessy™ Grading System, Model-GP7) was used to record the backfat thickness (BT) and *longissimus lumborum* (LL) depth, which were then used to estimate lean meat yield.

The LL pH was measured in the hot carcass 45 minutes after slaughter (pH45), and in the chilled carcass, which was kept in cold storage (1–2 ºC) for 24 hours (pH24), using a portable digital pH meter (Hanna™ Instruments Model HI 99163). The pH was measured at approximately 3 cm deep on LL of the left side of each carcass, between the 10th and 11th ribs (Bridi & Silva, 2009). The pH meter was calibrated before use to pH 7.01 and 4.01.

Twenty-four hours after slaughter, LL samples (2.5 cm thick of slices, estimated weight 100 g from, between the 14th and 18th vertebra were used to determine drip loss, thawing, cooking loss and colour, as described by Bridi & Silva (2009), using two repetitions per animal.

Six lightness measurements were used to evaluate colour (L*, a*, and b*) using a colorimeter (Konica Minolta™ Model CR400). The instrument was calibrated before each analysis with black and white tiles (X = 80.4, Y = 85.3, Z = 91.5) using illuminant D-65 with a 10° standard observer; and 4 auto-average. The

components, L* (lightness), a* (red-green), and b* (yellow-blue), were expressed in the CIELAB colour system.

Table 1 Ingredients, chemical and energetic composition of basal diet used for gilts (70–100 kg), as-fed basis

Item	Content (%)
Ingredient (%)	
Corn	78.52
Soybean meal	17.64
Limestone	1.16
Dicalcium phosphate	1.02
Sodium bicarbonate	0.46
Antioxidant[1]	0.010
Salt	0.040
Vitamin supplement[2]	0.050
Mineral supplement[3]	0.250
L-lysine HCl	0.365
DL-methionine	0.098
L-threonine	0.141
L-valine	0.013
Glutamic acid	0.135
Inert[4]	0.080
Tilosin phosphate	0.020
Total	100.00
Composition	
Dry matter(%)[5]	89.03
Metabolizable energy (Mcal/kg)[5]	3.230
Crude protein (%)[5]	15.10
Calcium (%)[6]	0.757
Available phosphorus (%)[6]	0.276
Sodium (%)[6]	0.160
Potassium (%)[6]	0.601
Chlorine (%)[6]	0.150
SID lysine (%)[7]	0.892
SID threonine (%)[7]	0.598
SID met + cys (%)[7]	0.535
SID tryptophan (%)[7]	0.140
SID arginine (%)[7]	0.841
SID valine (%)[7]	0.615
SID leucine (%)[7]	1.230
SID isoleucine (%)[7]	0.516
SID Phe + Tyr (%)[7]	1.094
RatioTrp : LNAA (%)[8]	3.704
RatioTrp : Lys (%)	15.70
B6 vitamin (mg/kg)[6]	1.000
DEB (mEq/kg)[9]	180.96

[1] BHT (butyl hydroxy toluene)
[2] Vitamins supplied per kilogram of diet: vitamin A, 5,000 IU as vitamin A acetate; vitamin D3, 750 IU as D-activated animal sterol; vitamin E, 15 IU as alpha tocopherol acetate; vitamin K3, 2.4 mg as menadione dimethyl pyrimidinol bisulphite; thiamin, 1.9 mg as thiamine mononitrate; riboflavin, 4.3 mg; vitamin B6, 0.29 mg as pyridoxine hydrochloride; vitamin B12, 0.027 mg; D-pantothenic acid, 17 mg as calcium pantothenate; niacin, 28 mg; folic acid, 0.6 mg; and biotin, 0.09 mg
[3] Minerals supplied per kilogram of diet: Cu, 25 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 0.31 mg as potassium iodate; Mn, 26 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 130 mg as zinc oxide
[4] Fine clean sand
[5] Analysed by AOAC (2007) methods
[6] Calculated from NRC (2012)
[7]The total amino acids of corn and soybean meal were determined at Evonik Industries and the standardized ileal digestible (SID) amino acid concentrations of these feedstuffs were estimated by using the SID coefficients proposed by NRC (2012)
[8] LNAA: large neutral amino acids (valine, isoleucine, leucine, phenylalanine and tyrosine)
[9] Dietary electrolyte balance

Cooked LL samples were submitted to shear force measurements (N) with a texturometer (Stable Micro System™ Model TA-Xt2i) fitted with a Warner–Bratzler shear force blade using the software texture expert exponent of Stable Micro Systems. For this purpose, six subsamples in cylindrical shape (diameter 1.27 cm) were taken from each sample longitudinally and in the direction of the muscle fibres, according to Ramos & Gomide (2007).

A 2 x 4 factorial arrangement of dietary SID Trp and B6 levels was analysed using PROC MIXED of SAS software (SAS Inst. Inc., Cary, NC, USA). Normality of distribution was tested using the Cramer-Von Mises and Levene test. Carcass traits, organ weights, abdominal fat and pork quality data were subjected to analysis of variance. The F test was applied for vitamin B6 levels. The degrees of freedom related to SID Trp levels were deployed in orthogonal polynomials to obtain regression equations. Slaughter weight was used as a covariate for carcass traits data.

The incidence of PSE meat was measured in percentage, and the data were processed for y = arc sin $\sqrt{PSE/n}$, assuming binomial distribution according to the methodology described by Haddad & Vendramim (2000). Probability values of P <0.05 were used as the criterion for statistical significance, and P <0.10 was considered a tendency.

Results

No interactions (P >0.05) between the dietary SID Trp levels and B6 supplementation were observed on the carcass traits (Table 2), organ weights and abdominal fat (Table 3). There was a trend (P = 0.064) for supplementary B6 (5 mg kg^{-1}) to increase the ham weight (Table 2), and there was a positive effect (P =0.002) of B6 supplementation (5 mg kg^{-1}) on the ham yield. Also, a linear increasing effect (P = 0.007) was observed of SID Trp levels on lean meat yield (Figure 1a), as explained by the equation Y = 47.5934 + 36.2037X (R^2 = 0.87).

The other parameters were not affected (P >0.05) by SID Trp levels or B6 supplementation. The relative organ weights and abdominal fat (Table 3) were not affected (P >0.05) by treatments.

No interactions (P >0.05) between SID Trp levels and B6 supplementation were observed for pork quality parameters (Table 4). The B6 supplementation (5 mg kg^{-1}) showed a minor reduction on pH24 (P = 0.010) which did not decrease sharply compared with the baseline B6 treatment (1 mg kg^{-1}). B6 supplementation also reduced cooking loss (P = 0.043) of LL (Table 4).

The LL lightness (Minolta L*) presented a tendency to decrease (P =0.061) as SID Trp levels increased in the diets (Figure 1-b), as explained by the equation Y = 57.8499 – 21.8360X (R^2 = 0.42). There was also a trend (P = 0.066) for SID Trp to affect the yellow-blue colour of LL (Minolta b*), revealing that the lowest value (4.74) could be achieved at 0.193% SID Trp by deriving the equation Y = 15.5510 – 111.998 + 290.066X^2 (R^2 = 0.99), as shown in Figure 1c.

In addition, there was a quadratic effect (P = 0.006) of SID Trp levels on the shear force, where the highest value (32.98 N) was obtained at 0.177% SID Trp, fitting the equation Y = – 44.8272 + 876.596X – 2468.97X^2 (R^2 = 0.99), as shown in Figure 1d.

Table 2 Carcass traits of gilts (n = 64) of 70 to 100 kg bodyweight fed diets with various levels of standardized ileal digestible (SID) tryptophan (Trp) and supplemental vitamin B6

Item	SID tryptophan (%)				B6 (mg/kg)		SEM[1]	P-value			
	0.140	0.167	0.194	0.221	1.00	5.00		B6xTrp	B6	Trp	
										Lin	Quad
Slaughter weight (kg)	99.34 ± 5.35	98.99 ± 6.02	99.62 ± 5.85	98.72 ± 5.99	99.17 ± 5.61	99.16 ± 5.91	0.722	0.223	0.176	0.246	0.696
Hot carcass yield (%)	83.21 ± 1.39	82.35 ± 1.03	82.32 ± 1.93	81.82 ± 1.91	82.44 ± 1.83	82.40 ± 1.48	0.207	0.830	0.921	0.218	0.645
Cold carcass yield (%)	80.64 ± 1.95	79.96 ± 1.59	79.87 ± 2.09	79.56 ± 2.05	80.20 ± 2.04	79.81 ± 2.81	0.240	0.791	0.374	0.398	0.685
Ham weight (kg)[2]	11.38 ± 0.68	11.07 ± 0.67	11.23 ± 0.76	10.90 ± 0.85	11.09^{B} ± 0.79	11.19^{A} ± 0.71	0.094	0.313	0.064	0.567	0.837
Ham yield (%)[2]	30.16 ± 1.83	30.20 ± 1.16	30.16 ± 1.74	29.94 ± 1.19	29.62^{B} ± 0.99	30.60^{A} ± 1.60	0.176	0.802	0.002	0.713	0.655
L. lumborum depth (cm)	4.60 ± 0.33	4.58 ± 0.54	4.15 ± 0.58	5.20 ± 0.59	4.62 ± 0.38	4.71 ± 0.62	1.616	0.398	0.304	0.384	0.897
Backfat thickness (cm)	1.70 ± 0.20	1.54 ± 0.27	1.49 ± 0.27	1.41 ± 0.29	1.51 ± 0.25	1.56 ± 0.27	0.507	0.187	0.453	0.709	0.625
Lean meat yield (%)[3]	52.70 ± 3.00	53.93 ± 2.41	53.94 ± 3.86	55.96 ± 4.38	54.35 ± 4.08	53.91 ± 3.12	0.451	0.132	0.576	0.007	0.609

[1] Standard error of mean
[2] Different letters in the same row are different by the F test at 5% probability
[3] Obtained by the Hennessy® Grading System (Model-GP7)

Table 3 Relative organ weights and abdominal fat of gilts (n = 64) from 70 to 100 kg bodyweight fed diets with various levels of standardized ileal digestible (SID) tryptophan (Trp) and supplemental vitamin B6

Item	SID tryptophan (%)				B6 (mg/kg)		SEM[1]	P-value			
	0.140	0.167	0.194	0.221	1.00	5.00		B6xTrp	B6	Trp	
										Lin	Quad
Spleen (%)	0.17 ± 0.03	0.19 ± 0.03	0.18 ± 0.05	0.19 ± 0.03	0.19 ± 0.04	0.18 ± 0.03	0.004	0.819	0.174	0.182	0.317
Liver (%)	1.48 ± 0.18	1.44 ± 0.15	1.52 ± 0.17	1.58 ± 0.23	1.48 ± 0.15	1.53 ± 0.21	0.023	0.139	0.657	0.196	0.319
Kidneys (%)	0.38 ± 0.04	0.37 ± 0.04	0.37 ± 0.04	0.37 ± 0.05	0.37 ± 0.04	0.38 ± 0.04	0.005	0.965	0.695	0.237	0.456
Abdominal fat (%)	1.08 ± 0.32	1.04 ± 0.29	1.17 ± 0.28	1.27 ± 0.29	1.17 ± 0.34	1.10 ± 0.26	0.038	0.978	0.256	0.113	0.378

[1] Standard error of mean

Table 4 Meat quality measured on the *longissimus lumborum* of gilts (n = 64) from 70 to 100 kg bodyweight fed diets with various levels of standardized ileal digestible (SID) tryptophan (Trp) and supplemental vitamin B6

Item	SID tryptophan (%)				B6 (mg/kg)		SEM[1]	P-value			
	0.140	0.167	0.194	0.221	1.00	5.00		B6xTrp	B6	Trp	
										Lin	Quad
pH 45	6.17 ± 0.33	6.32 ± 0.43	6.30 ± 0.42	6.24 ± 0.38	6.20 ± 0.41	6.31 ± 0.36	0.048	0.409	0.167	0.572	0.207
pH 24[2]	5.68 ± 0.31	5.78 ± 0.32	5.66 ± 0.25	5.59 ± 0.28	5.60^{B} ± 0.24	5.75^{A} ± 0.32	0.037	0.814	0.010	0.109	0.133
Drip loss (%)	4.39 ± 1.79	3.59 ± 1.86	3.85 ± 1.72	3.59 ± 1.48	3.83 ± 1.32	3.88 ± 2.04	0.213	0.430	0.910	0.247	0.502
Minolta L*	54.70 ± 2.41	54.96 ± 2.74	52.40 ± 3.11	53.59 ± 4.08	53.66 ± 3.78	54.15 ± 2.59	0.403	0.954	0.479	0.061	0.495
Minolta a*	7.92 ± 2.85	6.46 ± 1.08	7.03 ± 0.79	6.74 ± 1.03	6.84 ± 1.20	7.23 ± 2.09	0.213	0.433	0.364	0.122	0.179
Minolta b*	5.57 ± 0.91	4.91 ± 0.75	4.77 ± 0.73	4.96 ± 1.17	4.96 ± 1.02	5.14 ± 0.86	0.117	0.525	0.425	0.156	0.066
PSE (%)[3]	6.25 ± 3.12	6.25 ± 3.12	0.00 ± 0.00	12.50 ± 1.56	6.25 ± 3.12	6.25 ± 3.12	0.032	0.926	0.716	0.813	0.741
Thawing loss (%)	11.08 ± 3.35	9.64 ± 2.65	10.66 ± 2.19	10.03 ± 2.75	10.69 ± 2.71	10.01 ± 2.80	0.344	0.154	0.227	0.400	0.465
Cooking loss (%)[2]	30.72 ± 5.85	31.35 ± 5.02	33.73 ± 4.85	30.28 ± 5.88	32.56^{A} ± 5.28	30.47^{B} ± 5.51	0.682	0.153	0.043	0.816	0.148
Shear force (N)	29.03 ± 6.03	31.87 ± 6.49	31.97 ± 6.31	27.75 ± 6.38	31.18 ± 7.00	29.03 ± 5.72	0.794	0.442	0.182	0.486	0.006[5]

[1] Standard error of mean
[2] Different letters in the same row are different by the F test at 5% probability
[3] PSE: pale, soft and exudative; Not different by Tukey Test at 5% probability, with transformed data for y = arc sin √PSE/n

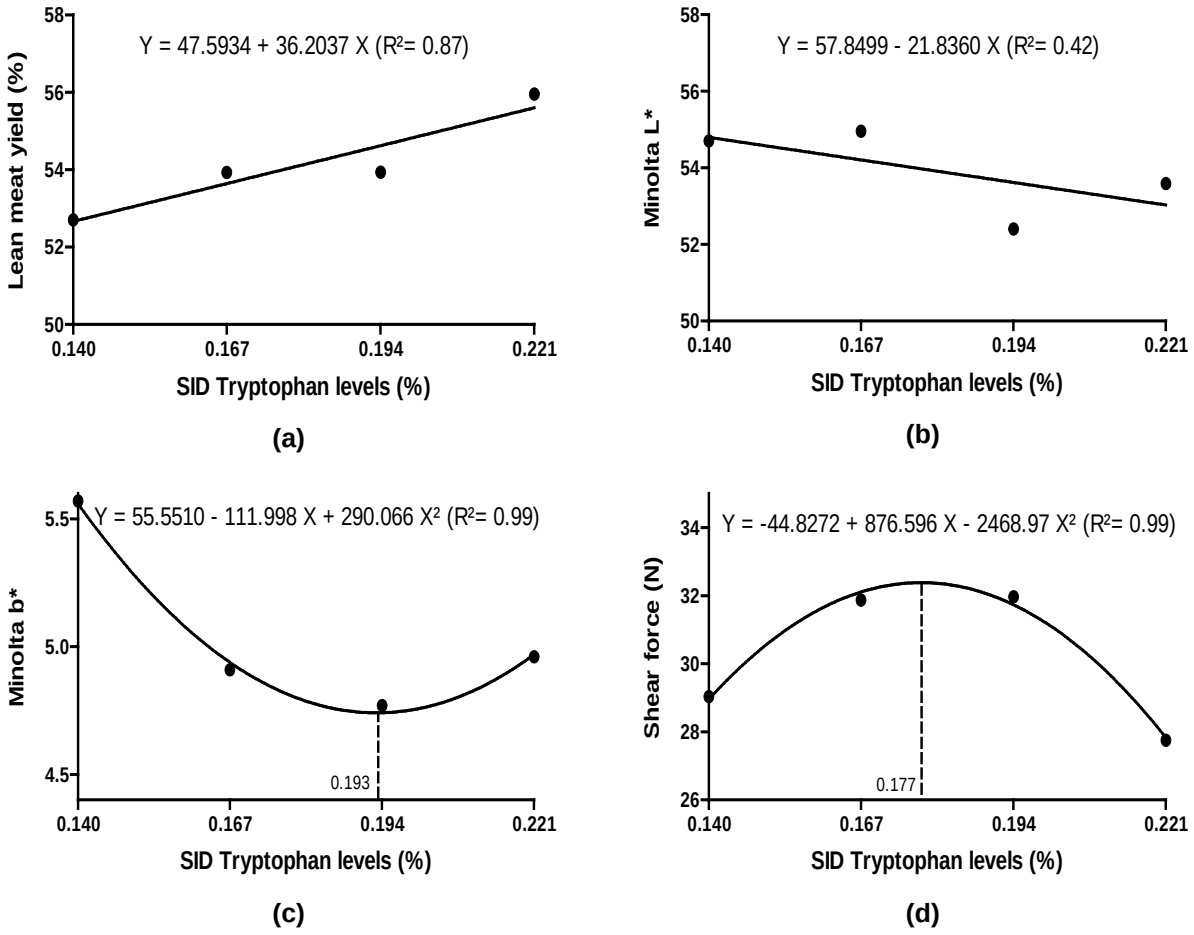

Figure 1 Effects of standardized ileal digestible (SID) tryptophan levels on lean meat yield (a), Minolta L* (b), Minolta b* (c) and shear force (d) of gilts from 70 to 100 kg bodyweight

Discussion

Dietary B6 supplementation did not provide an interaction with tryptophan on carcass traits (Table 2), as hypothesized earlier in this study and reported by Matte *et al*. (2011), owing to the action of B6 as a cofactor of many enzymes acting in Trp pathways. Nor was there interaction for organ weights and abdominal fat (Table 3).

Regarding carcass characteristics (Table 2), there was a positive effect of supplementary B6 (5 mg kg^{-1}) on ham yield (P = 0.002). This response may be associated with the trend (P = 0.064) observed for the ham weight. According to NRC (2012), to meet the deficiencies of vitamins in practical diets, vitamin premixes have been developed and are commonly added to pig diets. However, vitamin premix was used in the basal diet (as seen in Table 1), which supplied 1 mg kg^{-1} B6 in the diet, showing that pigs were responsive to a higher amount of B6 in the diet than currently used.

The results for ham weight and ham yield show a need to evaluate B6 levels in pig diets because it plays an important role in many metabolic pathways, not only with tryptophan. According to Nelson & Cox (2014), vitamin B6, as coenzyme pyridoxal phosphate (PLP), participates in enzymatic reactions related to the metabolism of carbohydrates and fatty acids, but mainly in reactions involving amino acid metabolism (transamination, decarboxylation, transsulfuration, and others), that could enhance protein deposition and meat yields.

The linear effect (P = 0.007) of SID Trp levels on lean meat yield may be due to the reduction on stress behaviour during the finishing phase (70 to 100 kg), which could have favoured protein deposition. Gilts fed with diets containing increasing levels of SID Trp probably presented reduced Trp competition with tyrosine for the same binding site on the blood brain barrier (Le Floc'h & Sève, 2007). Thus, a large amount of Trp may have been available for serotonin synthesis in the brain. In addition, increasing levels of Trp,

which might have mitigated tyrosine absorption in the small intestine, thus reducing the products of tyrosine (epinephrine and norepinephrine), which are responsible for the stress during the finishing phase and particularly at pre-slaughter, could not be released in sufficient concentrations to express animal stress. This could have improved anabolic processes and resulted in higher carcass and cut yields (Guzik *et al*., 2005).

Moreover, because of the sedative effect of a subsequent increased brain serotonin level, synthesized from Trp, a lowering effect on cortisol secretion might be expected (Henry *et al*., 1992), reducing the stress due to animal management. This effect may be related to the level and time at which animals are submitted. In the present work, animals were fed with experimental diets for about 26 days, and the highest mean value of lean meat yield (55.96%) was observed for the highest SID Trp level (0.221%), which means a difference of 3.26% in relation to the lower lean meat yield (52.70%) observed in the animals that received the lowest level of SID Trp (0.140%).

Even so, the linear increase of lean meat yield (P = 0.007) as dietary SID Trp levels increased was not observed by Haese *et al.* (2006) and Pereira *et al.* (2008), who observed no effect of SID Trp levels on lean meat yield for finishing pigs, but these authors used barrows. In a previous study, Castilha *et al.* (2016) showed no differences of increasing SID Trp levels for lean meat yield in 70–100 kg barrows receiving supplementary B6 in the diet.

Mammalian females are more sensitive than males to Trp deficiency, probably owing to differential distribution of serotonin in the brain, which is more localized (Henry *et al.,* 1996). Although barrows consume more feed and gain weight faster than gilts, they are more efficient in converting feed to weight gain, depositing a greater percentage of muscle tissue and reduced adipose tissue compared with barrows (Henry, 1995). It can explain in part the result obtained for lean meat yield in gilts.

Vitamin B6 supplementation (5 mg kg^{-1}) detained pH24 reduction (P = 0.010), which did not decrease sharply when compared with the average value of baseline B6 treatment (1 mg kg^{-1}), and reduced cooking loss (P = 0.043) of LL (as seen in Table 4). However, Kendall *et al.* (2007) evaluated SID Trp levels (from 0.06% to 0.16%) for 90–125 kg barrows and observed no effect on drip loss and cooking loss.

The pH24 and cooking loss are strongly related to each other and to the meat quality maintenance. The effect of B6 on these variables possibly occurred owing to the maintenance of glycaemic levels in the pre-slaughtering fasting period, which reduced the generation of lactate in the post-mortem period and consequently reduced the sudden drop in pH24 (Matarneh *et al*., 2015).

According to Combs Jr. (2008), B6 is essential in the maintenance of energy metabolism, in PLP form, especially in a situation of low glycaemia (fasting for example), because it is an enzymatic cofactor of glycogen phosphorylase, responsible for the cleavage of glycogen to release glucose (glycogenolysis), producing glucose-1-phosphate, which would be readily available to maintain glycaemia. This role of vitamin B6 in the animal organism accounts for the use of more than half of its total available concentration, owing to the abundance of the enzyme glycogen phosphorylase in muscles (about 5% of soluble muscle protein).

Post-mortem lactate accumulation in skeletal muscle is linearly associated with the extent of pH decline. Thus, maintenance of glycaemia in fasted animals was likely to be different for the group receiving supplemental B6 (5 mg kg^{-1}). This has reflected on pH24 owing to the reduction in lactate generation during the 24 hours post mortem.

In this work, increasing SID Trp levels presented a tendency to decrease (P = 0.061) LL lightness (Minolta L*) and there was also a trend (P = 0.066) of SID Trp to affect the yellow-blue colour of LL (Minolta b*). Even so, according to Bridi & Silva (2009) the L*, a* and b* values do not allow many conclusions when only one is studied, so these parameters must be associated with each other, or with other parameters to allow anomaly characterization in the flesh of pigs, especially PSE (light colour, soft texture and low water retention) and DFD (dry appearance, firm texture and dark colour) meats.

Thus, Bridi & Silva (2009) classify PSE meat as L* > 50, pH 45 ≤ 5.8, pH 24 ≤ 5.6, and drip loss > 5%; and DFD meat is classified as L* < 42, pH 24 > 6.0, and drip loss < 5%. Although the authors point out that all these characteristics must be considered so that anomalies (PSE or DFD) in the flesh can be determined, no effect was observed (P >0.05) for PSE (Table 4), but SID Trp levels affected the L* values linearly and the b* values quadratically.

There was a quadratic effect (P = 0.006) of SID Trp levels on the LL shear force. The highest value (32.98 N) was obtained at 0.177% of SID Trp. However, while the shear force indirectly represents the degree of meat softness, in the present study all values showed an extreme softness range, including the maximum value (31.98 N). According to Lyon & Lyon (1991), the LL of pigs can be rated in the same way as the *pectoralis major* of chickens. Thus, the authors ranked these samples as extremely soft (<35.50 N), moderately soft (35.51–64.82 N), slightly hard (64.83–94.14 N), moderately hard (94.15–123.56 N), and extremely hard (>123.56 N).

Overall, B6 and SID Trp levels recommended by the NRC (2012) for gilts from 75 to 100 kg are 1 mg kg^{-1} and 0.130%, respectively. However, from the results obtained in the present research one could

conclude that B6 supplementation (5 mg kg^{-1}) and SID Trp levels above nutritional requirements (0.140 to 0.221 %) provided consistent results in pork quality improvement and lean meat yield of gilts fed from 70 to 100 kg. Consequently, the authors believe there must be lower and greater B6 and SID Trp amounts in diets for gilts than they evaluated to bring about carcass traits and pork quality effects. This is an area that deserves further research.

Conclusion

In conclusion, vitamin B6 supplementation enhanced pork quality, and increased levels of SID tryptophan enhanced the lean meat yield when supplied in the diet of 70–100 kg gilts.

Acknowledgements

This study was funded by Fundação Araucária de Desenvolvimento Científico e Tecnológico do Estado do Paraná, Brazil.

Authors' Contributions

LDC and PCP designed this experiment. LACE, LPB, LMDH and MRF performed the animal experiment, measured and acquired the data. MSSP and ACF participated in result tabulation and statistics. LDC and PCP wrote the manuscript and revised it. MSSP, ACF and PCP supervised all processes through performing the experiment to writing the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest.

References

AOAC, 2007. Association of official analytical chemists. Official methods of analysis. 18th edition. Rev. 2. Gaithersburg, MD.

Bridi, A.M. & Silva, C.A., 2009. Avaliação da carne suína. 1st edition. Editora Midigraft, Londrina.

Cai, Y., Zimmerman, D.R. & Ewan, R.C., 1994. Diurnal variation in concentrations of plasma urea nitrogen and amino acids in pigs given free access to feed or fed twice daily. J. Nutr. 124, 1088-1093.

Castilha, L.D., Huepa, L.M.D, Fachinello, M.R., Pozza, M.S.S., Vasconcellos, R.S., Bonagurio, L.P., Marcato, S.M. & Pozza, P.C., 2016. SID tryptophan levels and B6 vitamin supplementation do not change blood parameters, organ weights, carcass traits, and meat quality of barrows (70-100kg). Meat Sci. 118, 66-70.

Combs Jr, G.F., 2008. The vitamins: Fundamental aspects in nutrition and health. 3rd edition. Elsevier Academies Press, Leiden.

Cromwell, G.L., 2004. Identifying the limiting amino acids in complex and cereal grain-based diets to minimize nitrogen excretion. In: Proceedings of the MidWest Swine Nutrition Conference. Ohio University Press, Columbus. pp. 69-83.

Guyton, A.C. & Hall, J.E., 2006. Medical Physiology. 11th edition Elsevier Saunders, Philadelphia.

Guzik, A.C., Shelton, J.L., Southern, L.L., Kerr, B.J. & Bidner, T.D., 2005. The tryptophan requirement of growing and finishing barrows. J. Anim. Sci. 83, 1303-1311.

Haddad, M.L. & Vendramim, J.D., 2000. Comparação de porcentagens observadas com caso extremos de 0 e 100%. Anais Soc. Entom. Bras. 29, 835-837.

Haese, D., Donzele, J.L., Oliveira, R.F.M., Abreu, M.L.T., Silva, F.C.O. & Saraiva, A., 2006. Níveis de triptofano digestível em rações para suínos machos castrados de alto potencial genético para deposição de carne na carcaça dos 60 aos 95 kg. Rev. Bras. Zoot. 35, 2309-2313.

Henry, Y., 1995. Effects of dietary tryptophan deficiency in finishing pigs: according to age or weight at slaughter or live weight gain. Livest. Prod. Sci. 73, 63-76.

Henry, Y., Sève, B., Colleaux, Y., Ganier, P., Saligaut, C. & Jégo, P., 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs in relation to plasma free amino acids and hypothalamic serotonin. J. Anim. Sci. 70, 1873-1887.

Henry, Y., Sève, B., Mounier, A. & Ganier, P., 1996. Growth performance and brain neurotransmitters in pigs as affected by tryptophan, protein, and sex. J. Anim. Sci. 74, 2700-2710.

Kendall, D.C., Gaines, A.M., Kerr, B.J. & Allee, G.L., 2007. True ileal digestible tryptophan to lysine ratios in ninety to one hundred twenty-five-kilogram barrows. J. Anim. Sci. 85, 3004-3012.

Kerr, B.J., McKeith, F.K. & Easter, R.A., 1995. Effects on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. J. Anim. Sci. 73, 433-440.

Le Floc'h, N. & Sève, B., 2007. Biological roles of tryptophan and its metabolism: Potential implications for pig feeding. Livest. Sci. 112, 23-32.

Le Floc'h, N., Matte, J.J., Melchior, D., van Milgen, J., Sève, B., 2010. A moderate inflammation caused by the deterioration of housing conditions modifies Trp metabolism but not Trp requirement for growth of post weaned piglets. Animal 4, 1891-1898.

Le Floc'h, N., Otten, W., Merlot, E. & Sève, B., 2011. Tryptophan metabolism, from nutrition to potential therapeutic applications. Amino Acids 41, 1195-1205.

Lewis, A.J. & Southern, L.L., 2000. Swine nutrition. 1st edition. CRC Press, Boca Raton.

Li, Y.Z., Baidoo, S.K., Johnston, L.J. & Anderson, J.E., 2011. Effects of tryptophan supplementation on aggression among group-housed gestating sows. J. Anim. Sci. 89, 1899-1907.

Li, Y.Z., Kerr, B.J., Kidd, M.T. & Gonyou, H.W., 2006. Use of supplementary tryptophan to modify the behaviour of pigs. J. Anim. Sci. 84, 212-220.

Lyon, B.G. & Lyon, C.E., 1991. Research note: shear value ranges by Instron Warner-Bratzler and single-blade Allo-Kramer devices that correspond to sensory tenderness. Poult. Sci. 70, 188-191.

Matarneh, S.K., England, E.M., Scheffler, T.L., Oliver, E.M. & Gerrard, D.E., 2015. Net lactate accumulation and low buffering capacity explain low ultimate pH in the longissimus lumborum of AMPKγ3 R200Q mutant pigs. Meat Sci. 110, 189-195.

Matte, J.J., Giguère, A. & Girard, C.L., 2005. Some aspects of the pyridoxine (vitamin B6) requirement in weanling piglets. Br. J. Nutr. 93, 723-730.

Matte, J.J., LeFloc'h, N., Primot, Y. & Lessard, M., 2011. Interaction between dietary tryptophan and pyridoxine on tryptophan metabolism, immune responses and growth performance in post-weaning pigs. Anim. Feed Sci. Technol. 170, 256-264.

NPPC, 2000. National Pork Producers Council. Pork composition and quality assessment procedures. Des Moines, Iowa.

Nelson, D.L. & Cox, M.M. 2011. Lehninger principles of biochemestry. 5th edition. Sarvier, São Paulo.

NRC. 2012. Nutrient requirements of swine. 11th edition. National Academy Press, Washington DC.

Pereira, A.A., Donzele, J.L., Oliveira, R.F.M., Abreu, M.L.T., Silva, F.C.O. & Martins, M.S., 2008. Níveis de triptofano digestível em rações para suínos machos castrados de alto potencial genético na fase dos 97 aos 125 kg. Rev. Bras. Zoot. 37, 1984-1989.

Poletto, R., Meisel, R.L., Richert, B.T., Cheng, H.W. & Marchant-Forde, J.N., 2010. Aggression in replacement grower and finisher gilts fed a short-term high tryptophan diet and the effect of long-term human–animal interaction. Appl. Anim. Behav. Sci. 122, 98-110.

Ramos, E.M. & Gomide, L.A.M., 2007. Avaliação da qualidade de carnes: fundamentos e metodologias. 1st edition. Editora UFV, Viçosa.

Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C., Oliveira, R.F., Lopes, D.C., Ferreira, A.S., Barreto, S.L.T. & Euclides, R.F., 2011. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3rd edition. Editora UFV, Viçosa.

Shen, Y.B., Voilqué, G., Odle, J. & Kim, S.W., 2012. Dietary L-tryptophan supplementation with reduced large neutral amino acids enhances feed efficiency and decreases stress hormone secretion in nursery pigs under social-mixing stress. J. Nutr. 142, 1540-1546.

Zhang, Z., Kebreab, E., Jing, M., Rodriguez-Lecompte, J.C., Kuehn, R., Flintoft, M. & House, J.D., 2009. Impairments in pyridoxine-dependent sulphur amino acid metabolism are highly sensitive to the degree of vitamin B6 deficiency and repletion in the pig. Animal 3, 826-837.