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N° 23

L-Tryptophan supplementation to enhance piglet growth

Tryptophan, like lysine, threonine and methionine is an essential amino acid. It has to be supplied through the feed, since its synthesis cannot be undertaken by the animal. Growing animals need tryptophan for protein deposition and also for various metabolic functions.

Unlike lysine which is mostly used for protein deposition (weight gain), tryptophan is involved in various metabolic pathways. The most important for pig production being appetite regulation. Tryptophan's role in growth and feed intake is well documented, but not necessarily quantified. The aim of this document is to highlight the benefits of manipulating tryptophan levels in piglet feed through L-Tryptophan supplementation.

Which tryptophan level in the feed to maximise piglet growth ?

The answer lies in the summation of several practical trials run in controlled environments at various experimental locations. Each trial presented here is a dose response trial where the only change in diet characteristics is L-Tryptophan addition. The incremental level of added L-Tryptophan leads to incremental levels of tryptophan in the feed while all other amino acids and nutrients remain the same.

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The tryptophan supply via the feed is expressed in relation to the lysine level, as a tryptophan to lysine ratio (Trp/Lys), where tryptophan and lysine are reported as ileal standardised digestible amino acids. Through reference to the ideal protein profile it allows the transfer of experimental findings into practical formulations where lysine specifications might differ from the ones chosen for the experiments (see info.2 at the end of the document). In each trial, the supply of amino acids other than tryptophan and lysine are set to be at the appropriate level or even in slight excess to avoid any limitation from factors other than the tryptophan itself. The composition of the experimental diets and the trial designs are detailed respectively in table 1 and table 2.

tab.1 - Experimental diets: composition and characteristics

	Schutte et al., 1989	Lynch et al., 2000	Jansman et al., 2000 a	Jansman et al., 2000 b	Pluske & Mullan 2000
Feed composition, %					
Wheat	-	10.0	55.0	-	32.3
Corn	15.0	48.6	5.0	53.0	34.0
Barley	35.0	5.0	-	-	-
Tapioca	13.4	-	12.9	8.2	-
Wheat middlings	2.0	-	-	-	-
Maize gluten meal	2.0	-	-	5.0	-
Meat and bone meal	1.0	-	2.0	2.0	-
Blood meal	-	-	-	-	1.1
Soybean meal	11.0	-	11.3	15.8	8.5
Soya whole seed	-	10.0	-	-	-
Pea	5.0	10.0	5.0	10.0	-
Lupin	-	-	-	-	4.5
Skimmed milk powder	-	-	-	2.0	4.0
Dried whey	-	5.0	2.5	-	5.2
Fish meal	5.6	9.8	-	-	5.5
Fat and/or oil	2.5	-	1.5	-	1.0
Others [1]	7.0	1.1	3.7	3.6	3.5
Amino acid supplementation, g/kg					
L-Lys HCl	2.9	2.8	5.7	2.8	3.4
L-Thr	1.0	1.9	2.3	0.6	1.4
DL-Met	1.1	1.4	2.6	1.4	0.6
Protein and total amino acid levels, % [2]					
CP	18.0 (18.1)	18.5 (18.2)	16.9 (17.2)	20.2 (20.7)	19.3 (19.1)
Lys	1.15 (1.15)	1.26 (1.22)	1.16 (1.23)	1.17 (1.24)	1.23 (1.23)
Thr	0.74 (0.75)	0.88 (0.87)	0.76 (0.76)	0.78 (0.81)	0.83 (0.83)
M+C	0.69 (0.70)	0.75 (0.72)	0.77 (0.75)	0.77 (0.79)	0.68 (0.68)
Trp	0.19 (0.19)	0.19 (0.18)	0.18 (0.18)	0.19 (0.20)	0.20 (0.20)
Digestible amino acid levels, % [3]					
Lys	1.04	1.12	1.06	1.05	1.12
Thr	0.64	0.77	0.67	0.68	0.73
M+C	0.62	0.67	0.70	0.70	0.61
Trp	0.16	0.15	0.15	0.16	0.17
Amino acid balance (digestible basis)					
Trp/Lys	16%	14%	15%	15%	16%
Thr/Lys	62%	68%	63%	64%	65%
M+C/Lys	60%	60%	66%	67%	55%
Trp/BCAA	6%	6%	6%	5%	6%
Trp/LNAA	4%	4%	5%	4%	4%

1 Others corresponds to minerals & vitamins, 2 Analysed value in brackets, 3 Ileal standardised digestibility (AmiPig 2000)
No antimicrobial growth promoters were added to the feed by Lynch et al. while it was the case for the feed made by the other authors

tab.2 - Experimental designs

	Schutte et al., 1989	Lynch et al., 2000	Jansman et al., 2000	Pluske & Mullan 2000
Number of piglets per pen	9	2	8	2
Number of pens per treatment	4	8	6	6
Number of piglets per treatment	36	16	48	12
Number of treatments	6	6	6	4
Total number of piglets in trial	216	96	288	48
Genotype*	LW x LD	(LW x LD) x meat line sire	(LD x GY) x GY	LW x LD
Sex	Males & Females in separate pens	1 male, 1 female per pen	Females	Entire males
Piglet weight	10 to 25 kg	11 to 29 kg	10 to 26 kg	7 to 16 kg

* LD: Landrace, Dutch Landrace in the case of Schutte et al. (1989) and Jansman et al. (2000), LW: Large White, GY : Great Yorkshire

- Schutte et al. (1989, The Netherlands) tested 6 different Trp/Lys ratios ranking from 16% to 24% (standardised digestible basis) on piglets from 10 to 25 kg live weight. Between the 2 extreme treatments the piglets exhibited a difference in weight gain of 20%. Feed conversion ratio (FCR) was also improved significantly but to a lower extent than the weight gain. The results are reported on table 3 and illustrated on figures 2 and 3 together with the results of the other trials.

- Lynch et al. (2000, Ireland) tested 6 Trp/Lys ratios from 15% to 23% on piglets from 10 to 30 kg live weight. The response to tryptophan level increase was almost linear, the highest weight gain being obtained when the Trp/Lys ratio is over 22%.

- Jansman et al. (2000, The Netherlands) tested 3 Trp/Lys ratios but on 2 different basal diets: one at 17% crude protein and the other at 20% crude protein. The 17%-protein diet corresponded to a lower level of Large Neutral Amino Acids (LNAA) together with a similar digestible tryptophan level as the other basal diet. The 17%-protein diet had then a higher Trp/LNAA ratio than the 20%-protein diet (table 1). This design aimed at verifying practically the relationship between Trp/LNAA ratio and feed intake (see details info.3). Energy, threonine, methionine and cystine supplies were the same across all the treatments. The choice of ingredients (table 1) had to be slightly different from the "low" to the "high" protein level, to match these numerous constraints without formulating a semi-synthetic diet.

  The higher the tryptophan level, the higher the piglet weight gain and the feed efficiency. The 17%-protein diet lead to significantly higher weight gain (+ 8%) than the 20%-protein diet, especially at high Trp/Lys ratio (figure 1). As

tab.3 - Piglet response to increased Trp/Lys ratios obtained with L-Trp addition (expressed as ileal standardised digestible amino acids)

Schutte et al., 1999	16%	17%	19%	20%	21%	24%
Starting weight, kg	9.5	9.5	9.5	9.5	9.5	9.5
Final weight, kg	22.6	24.4	25.1	25.6	26.0	26.1
Feed intake, g/d	782	870	902	926	932	923
Weight gain, g/d	468	532	556	575	588	594
FCR, kg/kg	1.67	1.64	1.62	1.61	1.59	1.55

Lynch et al., 2000	14%	15%	18%	21%	22%	23%
Starting weight, kg	10.8	10.5	11.0	10.9	10.9	10.8
Final weight, kg	25.8	28.9	29.5	30.0	30.6	30.4
Feed intake, g/d	876	931	944	950	975	1011
Weight gain, g/d	511	608	633	646	669	662
FCR, kg/kg	1.74	1.54	1.51	1.47	1.46	1.53

Jansman et al., 2000	17%, CP			20 %CP		
	15%	18%	21%	15%	19%	22%
Starting weight, kg	9.5	9.5	9.5	9.5	9.5	9.5
Final weight, kg	24.2	25.7	27.3	24.5	25.8	26.0
Feed intake, g/d	828	875	950	845	904	904
Weight gain, g/d	508	560	613	516	563	568
FCR, kg/kg	1.63	1.56	1.55	1.64	1.61	1.59

Pluske & Mullan, 200	16%	17%	19%	21%
Starting weight, kg	6.5	6.6	6.6	6.5
Final weight, kg	15.3	15.7	16.1	16.8
Feed intake, g/d	698	711	721	798
Weight gain, g/d	414	425	440	487
FCR, kg/kg	1.70	1.67	1.62	1.61

observed in other trials, weight gain is improved to a greater extent than the FCR by L-Tryptophan supplementation. It can thus be concluded from this trial that:

- enhanced weight gain obtained with Trp/Lys ratio above 20% is most likely due to an enhanced feed intake
- feed intake is specifically enhanced with diets having a high Trp/LNAA ratio meaning practically with balanced diets low in protein and high in tryptophan.

■ Pluske and Mullan (2000, Australia) worked with younger animals than the other authors and tested 4 levels of tryptophan ranking from 16% to 21% (Trp/Lys). As in the other trials, the higher the Trp/Lys ratio, the higher the weight gain. A difference of 14% in piglet weight gain was observed between the two extreme treatments.

fig. 1 Piglet weight gain as influenced by Trp/Lys ratio and diet protein level (Jansman et al., 2000, Trp/Lys expressed as ileal standardised digestible amino acids)

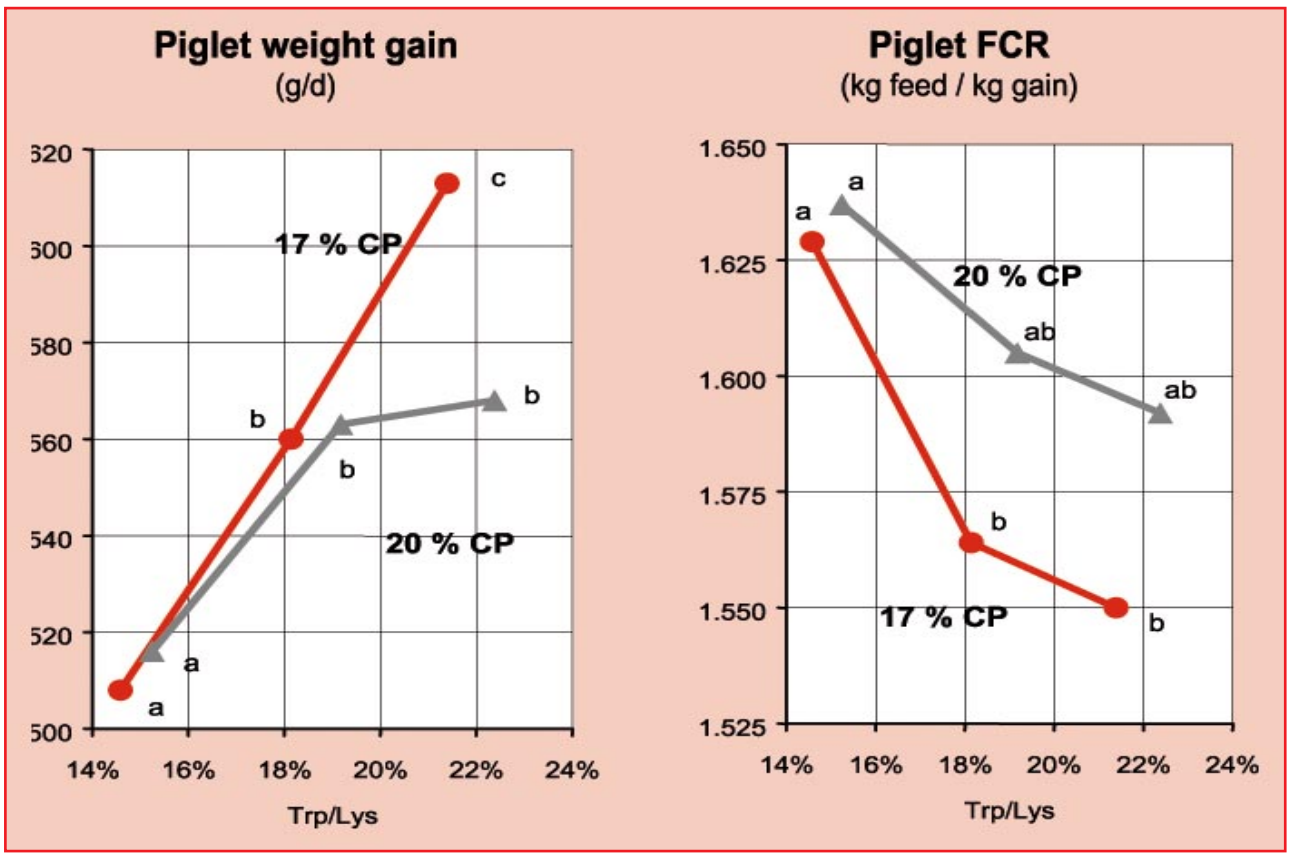


fig. 2 Trp/Lys impact on piglet weight gain

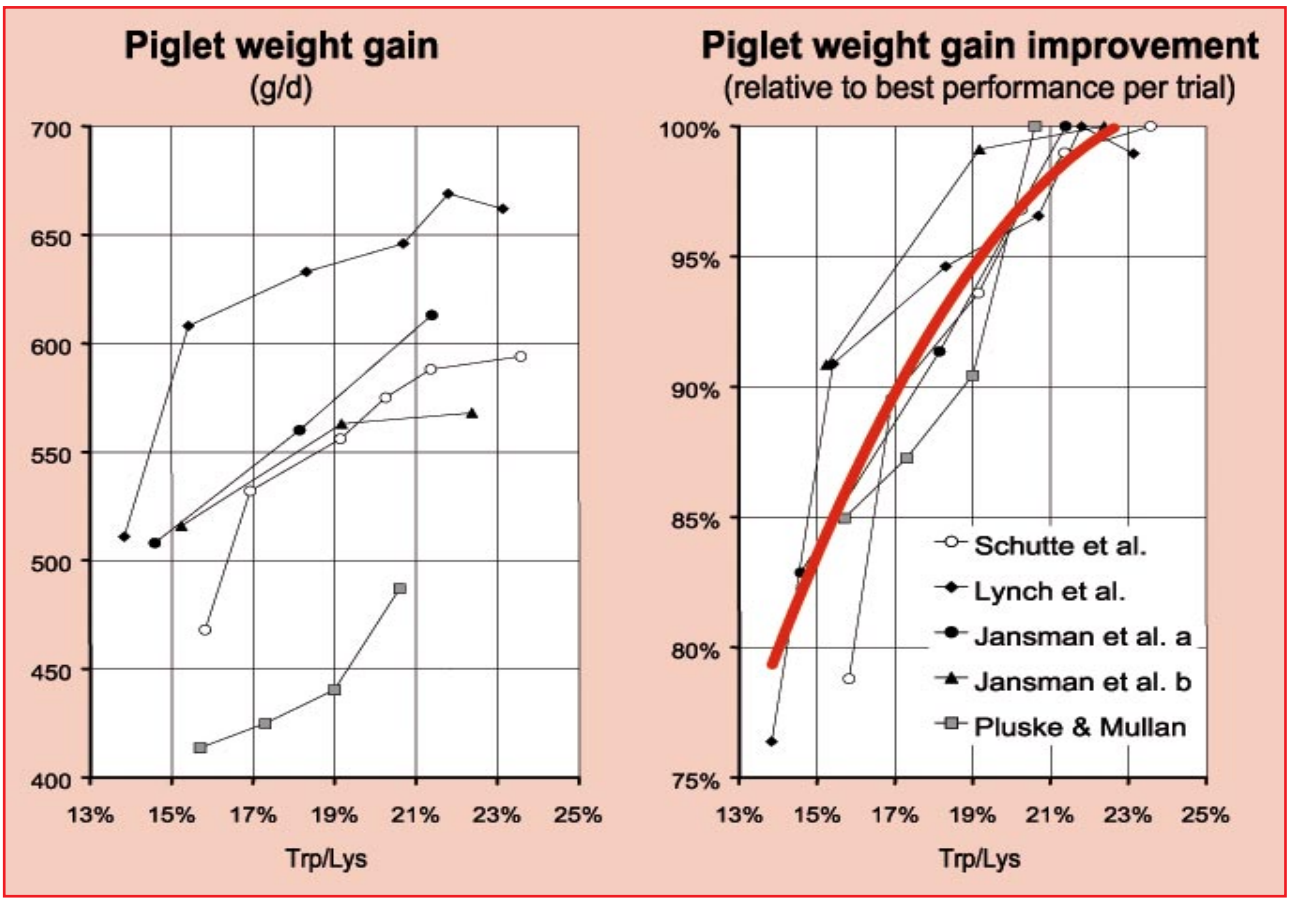
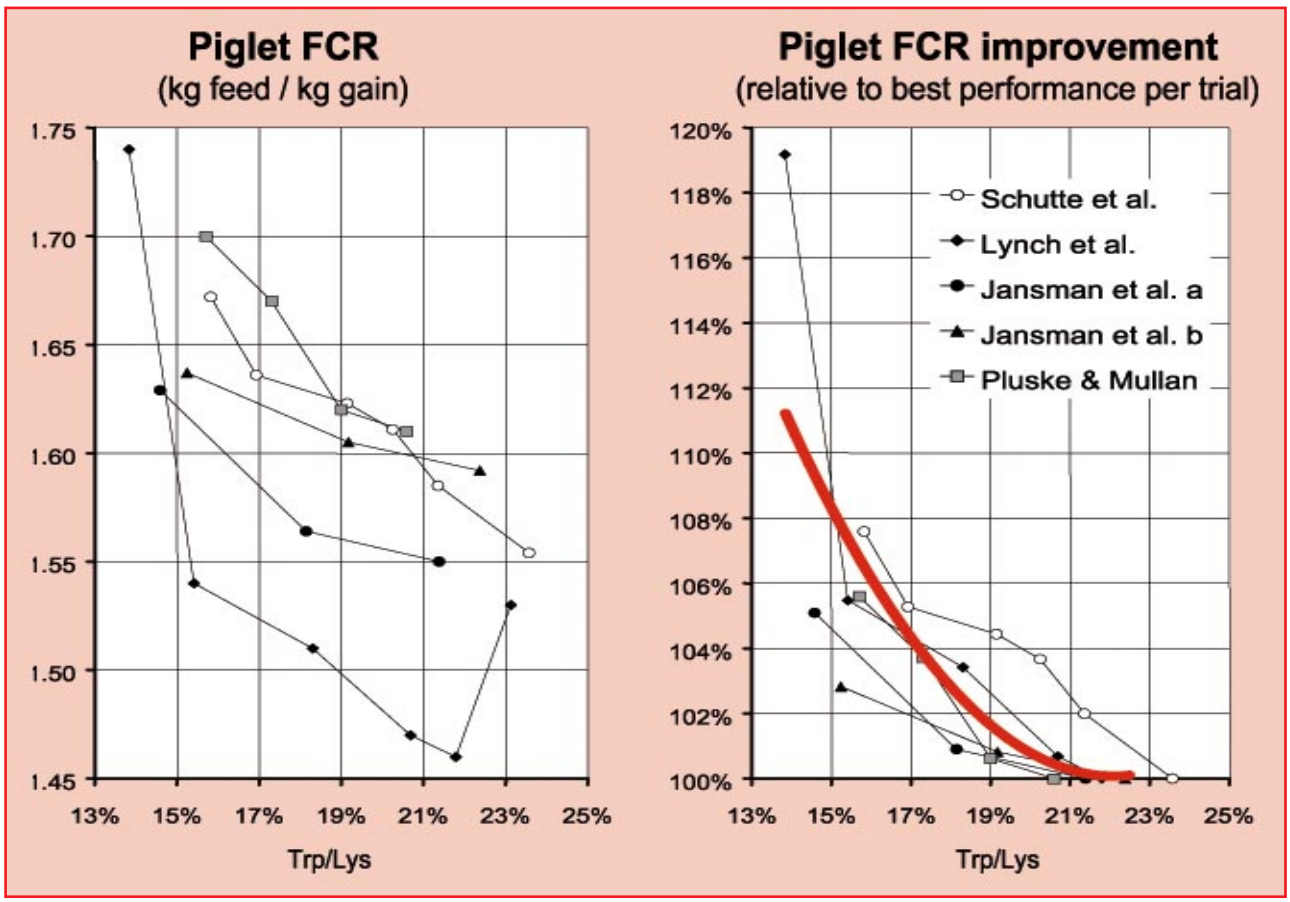


fig. 3 Trp/Lys impact on piglet feed conversion rate (FCR)



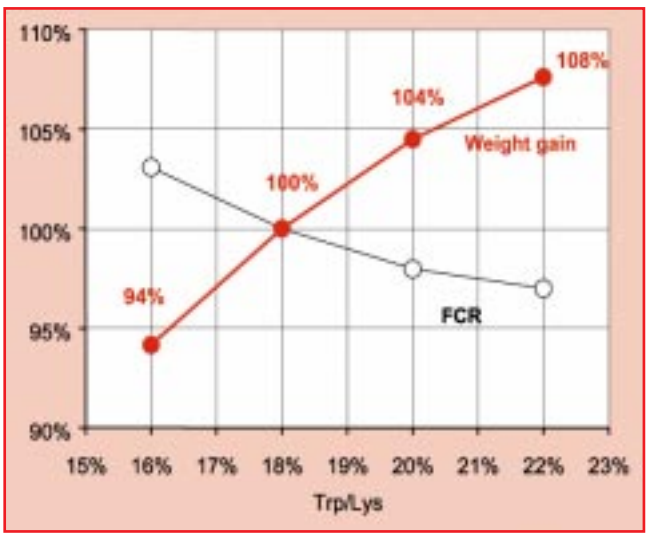
L-Tryptophan and piglet performance enhancement: consolidation of results

The consolidation of these experimental observations indicates the response trend to be expected from L-Tryptophan supplementation. A Trp/Lys ratio around 18% is acceptable for piglet growth but an increased growth is easily obtained by moving the Trp/Lys ratio to 22%. As shown above in addition to enhancing body protein deposition, tryptophan is likely to stimulate appetite, hence feed intake, and weight gain increases as a result. The information collected in these trials demonstrates that up-grading the Trp/Lys ratio from 18% to 22% increases piglet weight gain by 8% while improving on average the feed conversion ratio by 3% (figure 4).

The practical implications of increased weight gains

- **Enhanced growth:** thus a shorter period to grow the piglet to 25 or 30 kg. Days saved would vary between 1 and 3 depending on the Trp/Lys ratio used in the up-graded feed and the average weight gain normally seen on farm.

fig. 4 Piglet performance as a result of Trp/Lys manipulation with L-Trp addition (derived from figures 2 and 3)



As an example piglets gaining 450 grams of weight per day could reach 25 kg from 8 kg within 35 days instead of 38 days (3 days difference), if the Trp/Lys ratio is increased from 18% to 22% (provided no other factors restricts piglet growth potential).

- **Heavier piglet:** If the norm is to keep the piglet for a given time in the piglet unit then the farmer can achieve higher final weights. As an example, starting from 8 kg, piglets gaining 450 g/d can reach 28 kg within 40 days instead of 26 kg, thus a 2 kg advantage. In addition to a higher selling price for the piglet, heavier piglets are likely to be more amenable to environmental changes, transition to other feed, and go on to perform better in the follow on accommodations.

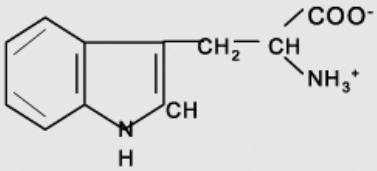
Conclusions

From the trial results reported in this document, it is concluded that piglet weight gain is maximised with a Trp/Lys ratio of 22%, while FCR is minimised with a slightly lower Trp/Lys ratio. The requirement for maximum protein deposition is linked with a requirement for maximum feed intake.

L-Tryptophan supplementation allows the tryptophan level in the feed to be set independently from that which can be supplied with current feedstuffs. It helps to maintain an adequate amino acid balance while formulating low protein diets (for sanitary and/or environmental reasons). L-Tryptophan may also confer further efficiency to low protein diets by enhancing piglets feed intake.

Info.1 L-Tryptophan molecule and product

tab.4. Feed grade, L-Tryptophan 98 % an Ajinomoto Eurolysine product

Molecule	L-Tryptophan content in product *	Regulatory position
	98%	EU Council Directive 82/471/CEE Position 3.4.1.

* commercial guarantee in product

Info.2 Setting the lysine level in a ratio dose response trial

Finding out what is the optimal Trp/Lys ratio necessitates a total use of the lysine offered in the diet. In case of excess lysine, the optimal tryptophan to lysine ratio is likely to be underestimated (all the tryptophan is used but not all the lysine, the ratio is then biased by a wrong lysine reference). On the other hand, in case of a lysine limitation the ratio found is still valid for a higher lysine level within the frame of the ideal protein concept.

The lysine level must not be particularly limiting and allow the expression of piglet potential for growth. Ideally lysine should be set at the appropriate level, to meet the exact animal requirement but this is not in reality possible for trials covering a growth period lasting from 10 to 20 kg or more. In ratio dose response trials lysine level is therefore voluntary set at a sub-limiting level, to ensure that the optimal tryptophan to lysine ratio corresponds to a maximum use of both lysine and tryptophan.

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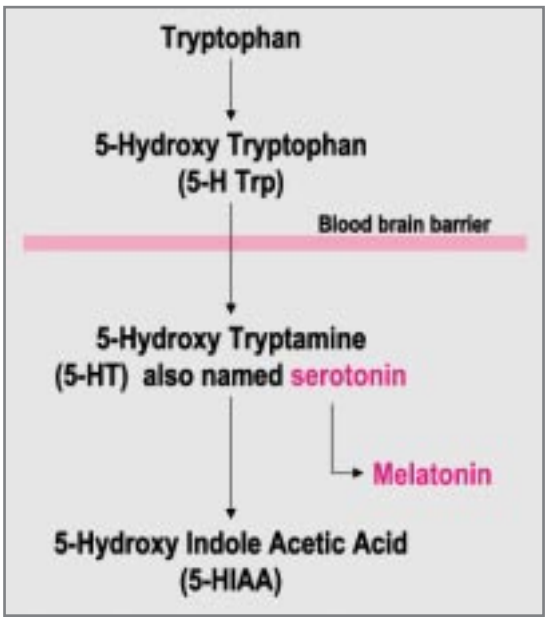
Info.3 Tryptophan impact on feed intake

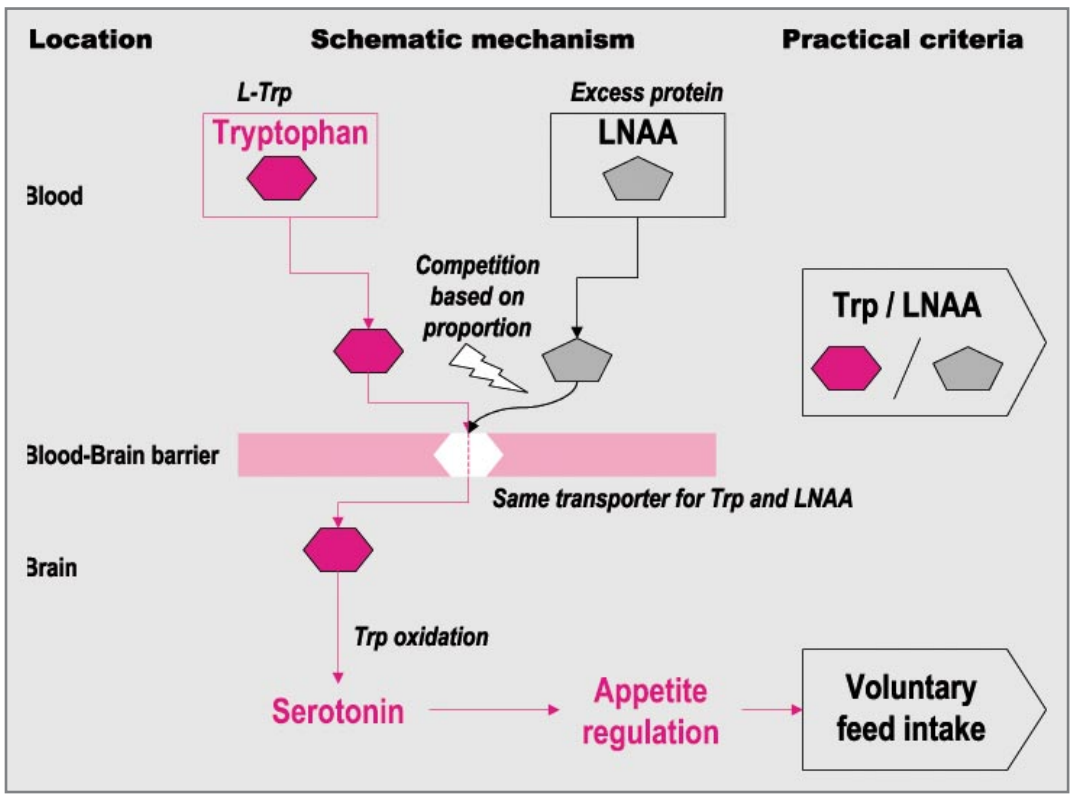
fig. 5 Schematic view of tryptophan conversion into serotonin. Though qualitatively of utmost importance this pathway is quantitatively of lesser importance than the other pathways.



One of the two oxidation pathways of tryptophan leads in the brain to serotonin production by the serotoninergic nerves (figure 5). Serotonin (5-HT), which has been shown to be directly proportional to plasma tryptophan, itself related to dietary supply, regulates several neurobehavioral effects like mood, pain perception, satiation and appetite. Favouring serotonin production through feed manipulation seems possible by providing the piglet with a "low protein – high tryptophan" diet. Indeed, as described in figure 6, serotonin production is conditioned by the proportion of tryptophan and Large Neutral Amino Acids (LNAA), namely Phenylalanine, Tyrosine, Isoleucine, Valine and Leucine. 5-Hydroxy Tryptophan and large neutral amino acids compete to enter the blood brain barrier via the same transporter system while only 5-Hydroxy Tryptophan leads to serotonin production, hence the importance of the Trp/LNAA ratio to possibly trigger piglet appetite. This mechanism was revealed in pigs by Henry et al. (1992) and seems to have been further demonstrated in practical diets by Jansman et al. (2000).

The only way to create diets with high Trp/LNAA is then to formulate diets with low protein level (low LNAA content) and high L-Tryptophan supplementation.

fig. 6 Interactive effects of tryptophan and large neutral amino acids (LNAA) resulting from excess protein on brain serotonin and appetite (adapted from Henry and Sève 1993)



Info.4 Digestible tryptophan content in various feedstuffs

Digestible tryptophan contents vary widely from one feedstuff to another (figure 7), as a result of the variability in total tryptophan content combined with the variability in tryptophan digestibility (figure 8). L-Tryptophan supplementation helps to overcome the variance in tryptophan supply from the feedstuffs, especially when formulating low protein diets.

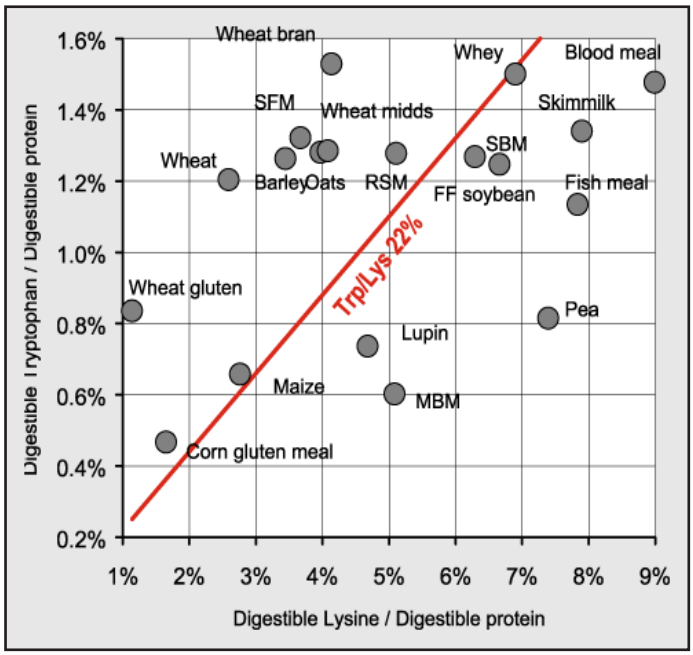
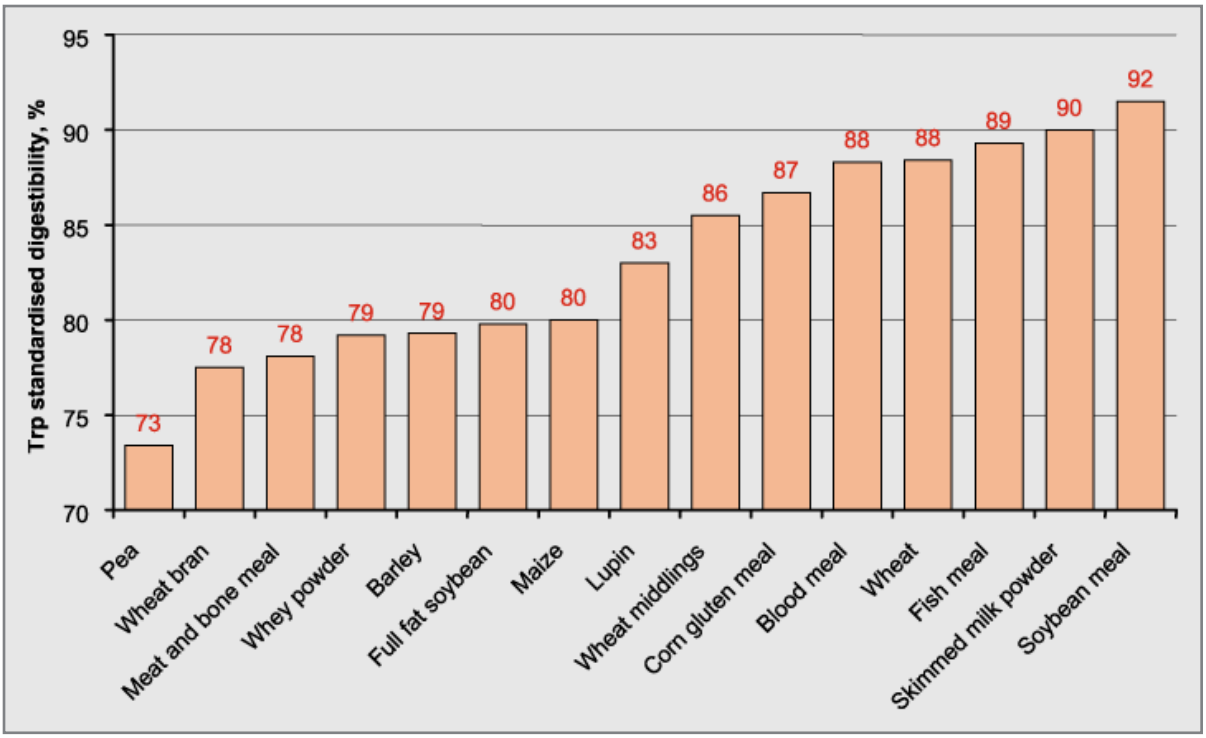


fig. 7 Digestible lysine and tryptophan profiles in some feedstuffs used in pig feeding. Feedstuffs below the red line show a Trp/Lys below 22% (SBM: Soybean meal, RSM: Rapeseed meal, SFM: Sunflower meal, MBM: Meat and bone meal, FF: Full fat Soybean)

fig. 8 Tryptophan ileal standardised digestibility in the feedstuffs used with in the aforementioned trials (AmiPig, 2000)



Info.5 Trp/Lys ratio as expressed in total or digestible amino acids

Trp/Lys ratio, from standardised digestible amino acids to total amino acids

Especially when free L-Lysine is added to the diet, lysine digestibility appears higher than that of tryptophan hence the shift between the different Trp/Lys ratio when expressed as total basis or in digestible terms (ratio in digestible basis < ratio in total basis, tab.5). However as soon as some L-Tryptophan is added to the diet the spread between the 2 ratios (total and digestible) tends to disappear due to the enhancement of tryptophan digestibility obtained with L-Tryptophan supplementation.

Trp/Lys ratio, from standardised digestible amino acids to apparent digestible amino acids

There is no such shift between the ratio expressed as standardised digestible amino acids or as apparent digestible amino acids as there is for the threonine to lysine ratio. Indeed the basal endogenous losses are not as high in tryptophan as in threonine which makes the ratio to lysine expressed as "apparent" or as "standardised" digestible amino acids very similar (tab.5).

tab.5. Example of a classical shift in Trp/Lys ratio when expressed in total or digestible terms (apparent or standardised) and according to the L-Tryptophan supplementation

	Total	Digestible (ileal standardised)	Digestible (ileal apparent)
Without L-Tryptophan	17%	16%	16%
With L-Tryptophan	22%	22%	22%

Info.6 Tryptophan analysis, intra-lab reproducibility

Tryptophan content in feed and feedstuffs is determined by High Pressure Liquid Chromatography (HPLC) on an hydrolysed solution of feedstuffs protein (alkaline hydrolysis in order to prevent tryptophan destruction). Tryptophan analysis (described in the Commission Directive 2000/45/EC, 6 July 2000) appears as repeatable (intra laboratory) and as reproducible (inter laboratory) as the analysis for other amino acids (tab.6).

tab.6. Reproductibility and repeatability of total tryptophan analysis

	Pig feed (1)	Pig feed with L-Trp added (1)	Pig feed concentrate (1)	Pig feed (2)	Soybean meal (2)	Pea (2)
L	12	12	12	1	1	1
n	50	55	50	10	10	10
Mean, g/kg	2.42	3.40	4.22	2.46	6.00	1.89
s_r	0.05	0.05	0.08	0.03	0.10	0.04
CV_r	1.9%	1.6%	1.9%	1.3%	1.6%	1.9%
s_R	0.15	0.2	0.09			
CV_R	6.3%	6.0%	2.2%			

L: number of laboratories submitting results, n: number of single results, s_r: standard deviation of repeatability, CV_r: coefficient of variation of repeatability, s_R: standard deviation of reproducibility CV_R: coefficient of variation of reproducibility

(1) From the Commission directive 2000/45/EC 6 July 2000

(2) Ajinomoto Eurolysine internal evaluation, the 10 analyses were performed during a 7 month period

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