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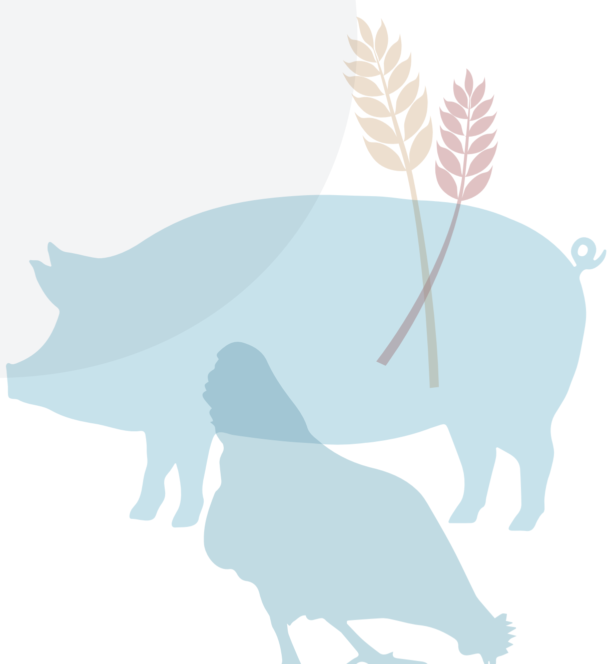

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Importance of tryptophan in swine fed with animal by-products

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INTRODUCTION

Use the nutritional recommendations within each swine phase, know the digestibility coefficient of protein and amino acids in each raw material, then applying the ideal protein concept and, take advantage of industrial amino acids, nutritionists can formulate lower cost diets with an adequate energy level, essential and conditionally essential nutrients for maximum genetic expression of animals and with minimal environmental effect.

With the diversity of raw materials available for formulation in swine diets, limiting amino acids are those that are present in the diet in a concentration lower than the requirement for the maximum growth for swine. Limiting amino acids are recognized by their order of limitation by giving a chemical score in formulated diet. Also, the limitation order is in accordance with the age of the animal or physiological state. However, the first limiting amino acid in swine will typically always be lysine (Lys) (Henry et al., 1992; Bertechini, 2012). In a diet based on corn and soybean meal for swine, tryptophan is considered the fourth or fifth limiting amino acid after Lysine, Met + Cys and Threonine (Bertechini, 2012 and Pereira, 2014). On the other hand, in countries that allow the use of animal by-products (meat and bone meal, feather meal and gut meal) and, depending on the level used in the diet, the amino acid tryptophan can become the second (Lima et al., 2012) or third limiting, thus requiring dietary supplementation to meet the animal's requirement.

The objective of this article is to show that tryptophan is not only as an essential and limiting amino acid for swine fed diets containing animal by-products, but also the importance of the tryptophan:lysine digestible ratio (Trp:Lys SID) on physiological potential, performance response, animal health and welfare.

Importance of tryptophan in swine diet

The essentiality of tryptophan determines its importance in the deposition of tissues, reflecting on the efficiency of animal growth. Also, this amino acid is involved in the synthesis of niacin (vitamin B3) and melatonin (neurohormone). Tryptophan is an important immune system intermediary as its requirement seems to increase during an inflammatory response (Pereira, 2014).

According to Henry et al. (1992) marginal tryptophan needs should not be overlooked in feed formulations, as this amino acid is a precursor to serotonin in the brain (5-hydroxytyryptamine), a neurotransmitter involved in regulating feed consumption. Serotonin is directly related to mood, behavior and cognition (Richard et al., 2009) which can be directly linked to animal welfare.

In an experiment carried out by Adeola and Ball (1992), it was observed that the maximum serotonin concentration in the swine hypothalamus during the finishing phase occurred in 5 days after the supplementation of tryptophan in the diet (Fig. 1). Also, they found that supplementing tryptophan in the pre-slaughter period can reduce the stress response and decrease the PSE pork.

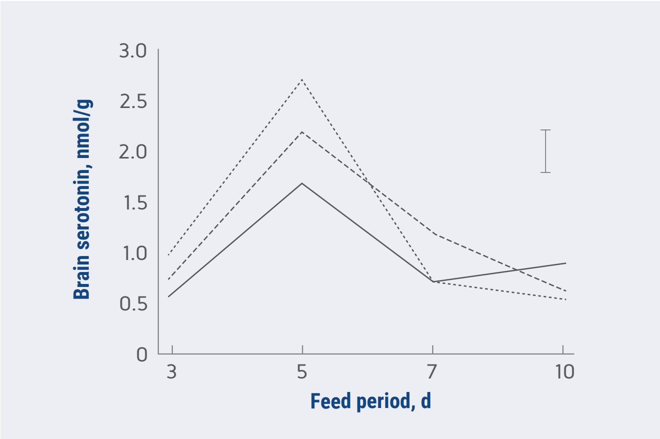


Figure 1. Hypothalamic serotonin concentration in pigs offered supplemental tryptophan at 2.5 (—), 5 (---) or 10 (···) g/kg of diet for 3, 5, 7 or 10 days. The vertical bar represents standard error of the mean.

(Adopted from Adeola and Ball, 1992)

Another important evidence of tryptophan is its direct relationship with the synthesis of ghrelin, a hormone which is present in the stomach, intestine and other organs that impacts appetite in cooperation with the nervous system. It also increases Growth Hormone synthesis (Correia-Silva, 2008).

Zhang et al. (2007) conducted a study to determine whether ghrelin produced mainly by the stomach, was involved in tryptophan-mediated appetite stimulation in swine. They used diets based on corn, corn gluten and soybean meal containing 3 increasing levels of supplemental tryptophan (0.12%; 0.19% and 0.26% in the diet or 0.0 g/kg; 0.07 g/kg and 0.14 g/kg of complete feed); providing Trp:Lys SID ratios of 10%; 15.7% and 21%. The diets were offered *ad libitum* versus limited fed (feeding manner). Weight gain, feed intake and feed conversion were all improved with increased ingestion of dietary tryptophan (Table 1). Dietary tryptophan induced higher ghrelin mRNA levels (P<0.01), but feeding manner (P>0.05) had no effect on the expression of ghrelin in gastric fundus in weanling pigs (Fig. 2A). Feeding 0.19% tryptophan diet induced the higher level of mRNA of ghrelin among the three dietary treatments in two feedings manners. In duodenum tissue, ghrelin mRNA levels was augmented by tryptophan concentration in diet (P<0.01), the 0.26% tryptophan diet induced the highest ghrelin levels in duodenum (Fig. 2B).

Table 1. Effect of dietary tryptophan level on the performance of weanling piglet

(Adopted from Zhang et al., 2007)

Parameters	Feeding manner			Dietary tryptophan, %			
	Limited fed	Ad libitum	S.E.M	0.12	0.19	0.26	S.E.M
WG, g/d	357	487*	10.68	365^{a}	435^{ab}	465^{b}	13.08
FI, g/d	484	704*	13.01	556^{a}	594^{ab}	632^{b}	16.03
FGR	1.37	1.46	0.02	1.53^{a}	1.36^{b}	1.35^{b}	0.02

Means in the same row followed by the different letters are significantly (P<0.05)
WG – weight gain; FI - feed intake; FGR - feed:gain ratio

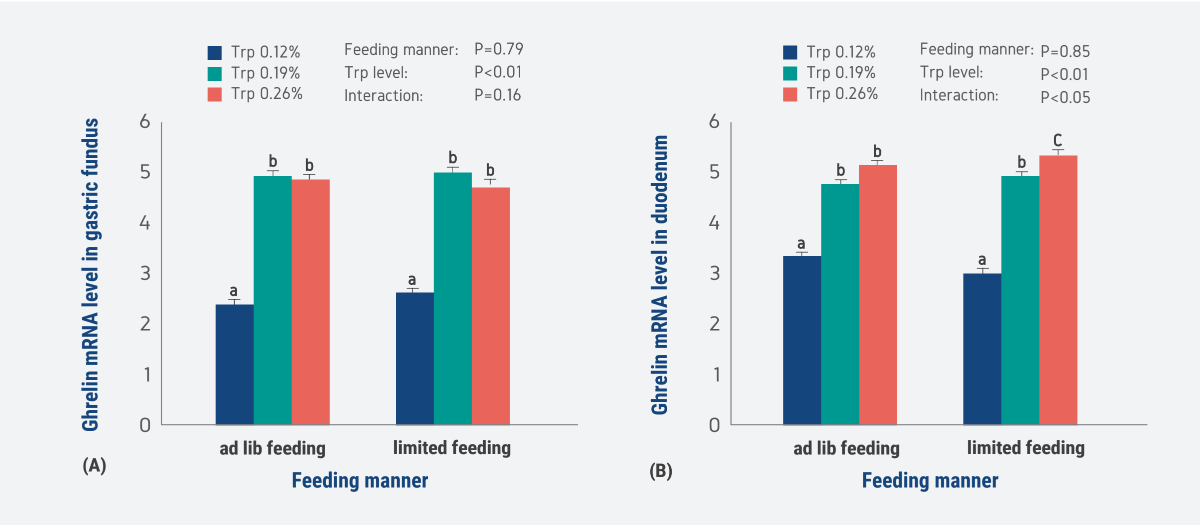


Figure 2. Effects of feeding manner and dietary tryptophan levels on ghrelin mRNA levels in gastric fundus (A) and duodenum (B) in weanling piglet. Ghrelin mRNA levels are mean±S.E.M. Bars with different letters mean significantly different (P<0.05).

Tryptophan and large neutral amino acids (leucine, isoleucine, valine, phenylalanine and tyrosine) compete at the blood-brain barrier, as they share a common transport system. Given this circumstance, the amount of tryptophan in the brain can be reduced if the intake of large neutral amino acids (LNAA) is high (Pereira, 2014). This can negatively impact the synthesis of serotonin. Understanding the relationship between tryptophan/LNAA can be an important factor in the formulation of diets to diagnose the best mechanism for feed consumption, improved health and welfare of swine. In the review carried out by Adeola and Ball (1992), there is evidence that the increasing tryptophan relative to the other LNAA improves the level of serotonin in the brains of rats, broilers and layers causing a sedative effect.

The facts above can be considered crucial when nutritionists are faced with high availability of meat and bone meal, gut meal and feather meal from slaughterhouses and rendering industries because tryptophan can be the second limiting amino acid in swine diets (Lima et al., 2012).
The Trp:Lys SID ratios for swine is referenced by Rostagno et al. (2011) who recommend 18% for the initial, growth and finishing phases. Rostagno et al. (2017) establish 19% for the initial phase and 20% for the growth and finishing phases. For the NRC (2012) the recommendation is 16% for the initial phase and 17% for the growth and finishing phases (barrows, females and entire males).

Animal by-products in the swine diet

Rendering industries in different parts of the world have strict governmental regulations, good manufacturing processes and controls leading to high quality products. This is an important link for sustainable animal production. They are recycling companies and essential for the environment safety, public and animal health (Juzefowicz, 2017).
The production, quality and safety specifications for animal by-products are well documented in the literature (Compendium, 1998; Bellaver, 2001; Bellaver, 2002), being an excellent alternative to reduce the final cost of a diet in view of the high price soybean meal and phosphate (Thaler and Holdon, 2010; Pereira, 2014). It is up to the nutritionists to know the practical and maximum inclusion of animal by-products for swine (Table 2).

Table 2. Inclusion of animal by-products for swine (%)
(Adopted from Rostagno et al., 2011)

Raw Materials	Swine					
	Starter		Grower		Finisher	
	Practical	Maximum	Practical	Maximum	Practical	Maximum
Feather meal	1%	2%	2%	4%	2%	5%
Feather and gut meal	1%	2%	2%	4%	2%	5%
Gut meal for poultry	3%	5%	4%	7%	4%	8%
Gut meal for swine	3%	5%	4%	6%	4%	7%
MBM (41%)	3%	5%	4%	6%	4%	7%
MBM (50%)	4%	6%	4%	7%	4%	8%
Blood meal	1%	2%	1%	3%	2%	4%

The content of calcium and phosphorus present in meat and bone meal may be the limiting factor for its inclusion in the swine diets. Also, it is recommended that the total amount of animal by-products should not exceed 10% to 12% of the diets in grower and finisher phases. In an experiment carried out by Lima et al. (2012) with the objective of evaluating Trp:Lys SID ratios in finishing pig phase (70 to 95 kg), 8% of gut meal and 2% of feather meal had no negative impact on the final performance of animals.
A crucial point for the successful use of animal by-products in swine is to know the content and digestibility of each nutrient, due to variation in the crude protein and amino acid content with the lack of standardization of raw materials (Pereira, 2014).

Effect of tryptophan on swine fed on diets containing animal by-products

In order to understand the effect of tryptophan on swine fed diets containing animal by-products, a research project was carried out by Pereira (2014) at the Federal University of Viçosa in Brazil. To assess the reduction of soybean meal by use of animal by-products in swine fed with two Trp:Lys SID ratio. Pereira (2014) used 96 barrows (PIC x PIC hybrids) in the starter phase (15 to 25 kg), grower phase (30 to 65 kg) and finishing phase (70 to 95 kg). The animals were distributed in a randomized block design, blocked on initial weight (light, medium and heavy), in a 2 x 2 factorial experiment (protein source: vegetable or animal by-products); Trp:Lys SID ratios (18 and 21%) with 12 replicates of two animals each. The experimental diets (Table 3) were formulated to be isonutritive and meet or exceed the recommendations of Rostagno et al. (2011). The inclusion of meat and bone meal and feather meal was observed at the practical levels recommended by Rostagno et al. (2011).
For the 21% Trp:Lys SID ratio, L-Tryptophan replaced starch in the basal diets containing either vegetable or animal by-products. Diets and water were provided *ad libitum* throughout the experimental period. The final weight, daily feed intake, daily weight gain and feed efficiency ($p < 5\%$) were determined by SAS.

Table 3. Composition of experimental diets

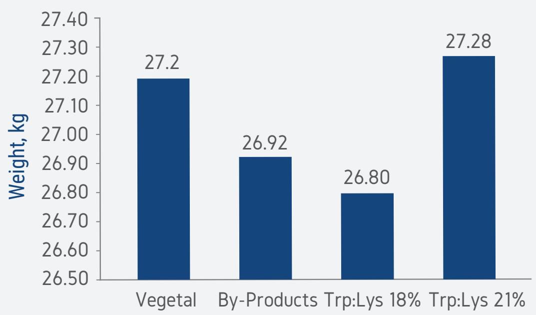
(Adopted from Rostagno et al., 2011)

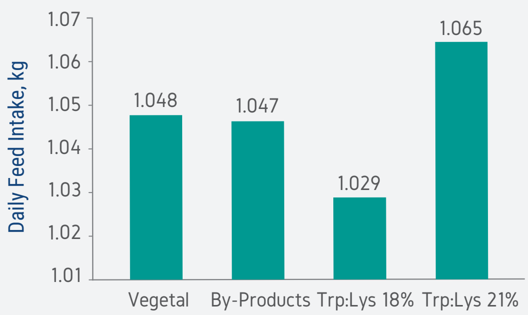
Ingredients	18% Trp:Lys SID					
	15 -25 kg		30 - 65 kg		70 - 95 kg	
	Vegetal,%	By-products,%	Vegetal,%	By-products,%	Vegetal,%	By-products,%
Corn	68.95	72.35	75.08	78.13	80.42	81.41
Soybean meal, 45%	25.70	19.30	20.40	13.60	15.60	10.90
MBM, 44%	--	3.00	--	3.50	--	3.50
Feather meal, 84%	--	1.50	--	2.00	--	2.00
Soybean oil	2.20	1.90	1.80	1.50	1.50	1.30
Dicalcium phosphate	0.958	0.072	1.044	--	1.065	--
Limestone	0.960	0.580	0.590	0.150	0.440	--
Salt	0.460	0.390	0.390	0.320	0.350	0.350
L-lysine	0.295	0.400	0.260	0.355	0.230	0.260
DL-methionine	0.105	0.100	0.085	0.065	0.055	0.033
L-threonine	0.060	0.070	0.045	0.045	0.030	--
L-tryptophan	--	0.025	--	0.025	--	0.010
Premix vitamin	0.100	0.100	0.100	0.100	0.100	0.100
Premix mineral	0.100	0.100	0.100	0.100	0.100	0.100
Starch	0.100	0.100	0.100	0.100	0.100	0.100
Antioxidant	0.010	0.010	0.010	0.010	0.010	0.010
Total	**100**	**100**	**100**	**100**	**100**	**100**
Value Calculated						
Crude protein, %	17.4	17.4	15.45	15.89	14.18	14.79
ME, kcal/kg	3320	3320	3320	3320	3320	3320
Ca, %	0.720	0.720	0.591	0.591	0.529	0.529
P, %	0.350	0.350	0.358	0.358	0.355	0.355
Na, %	0.200	0.200	0.175	0.175	0.160	0.160
Lys, SID, %	1.006	1.006	0.855	0.855	0.718	0.718
Met, SID, %	0.342	0.331	0.303	0.276	0.252	0.233
Met+Cys, SID, %	0.604	0.604	0.539	0.539	0.468	0.485
Thr, SID, %	0.634	0.634	0.556	0.556	0.481	0.481
Trp, SID, %	0.181	0.181	0.154	0.154	0.129	0.129
Val, SID, %	0.724	0.723	0.641	0.641	0.562	0.618
Leu, SID, %	1.411	1.381	1.298	1.288	1.191	1.233
Ile, SID, %	0.647	0.608	0.561	0.533	0.481	0.490

SID - standardized ileal digestibility.
Diet 15 - 25 kg: - 21% tryptophan: lysine DIE ratio, starch was replaced by 0.03% L-Tryptophan.
Diet 30 - 65 kg: - 21% tryptophan: lysine DIE ratio, starch was replaced by 0.025% L-Tryptophan.
Diet 70 - 95 kg: - 21% tryptophan: lysine DIE ratio, starch was replaced by 0.022% L-Tryptophan.

Results

In the starter phase, there was no interaction (P> 0.05) of protein sources and the Trp:Lys SID on weight, daily feed intake, daily weight gain and feed efficiency (Fig. 3).





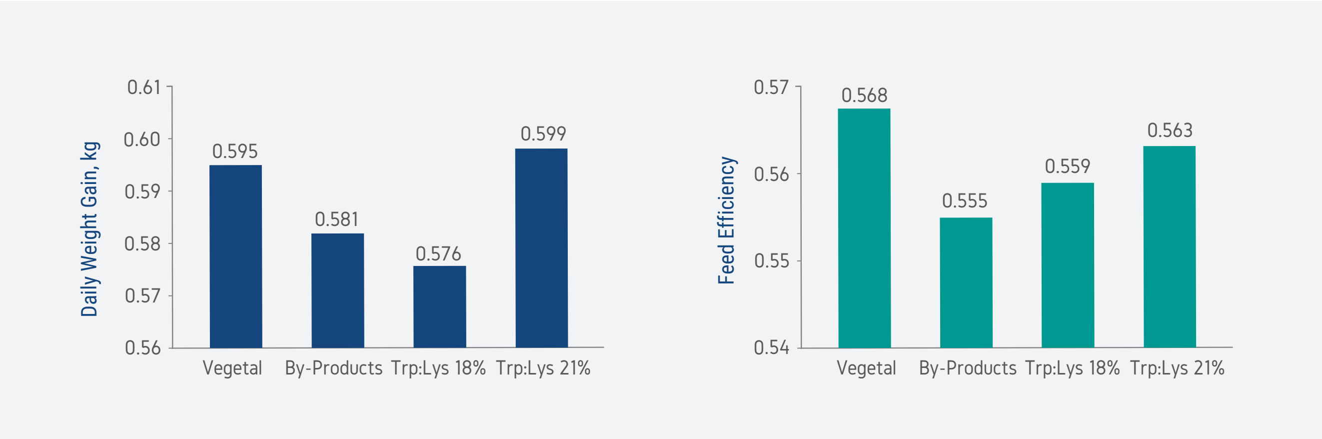


Figure 3. Performance of swine from 15 to 25 kg fed with two protein sources and two Trp: Lys SID ratios (P> 0.05).

For swine weighing 30 to 65 kg (Table 4), there was no interaction between the treatments. Pigs fed diets containing animal by-products had lower daily feed intake (P <0.05). The two protein sources did not influence the final weight, daily weight gain and feed efficiency. On the other hand, swine fed with Trp: Lys SID ratio of 21%, regardless of protein sources, had a higher (P <0.01) final weight, daily weight gain and feed efficiency. No difference in daily feed intake was found between the two Trp:Lys SID ratios. For swine weighing 70 to 95 kg, there was no interaction between protein sources and Trp:Lys SID ratios on animal performance. Protein sources did not affect the final performance of animals. However, pigs fed with Trp:Lys SID ratio of 21%, had greater weight gain, daily feed intake and daily weight gain. Trp:Lys SID ratios did not affect the feed efficiency (Table 5).

Table 4. Performance of swine from 30 to 65 kg fed with two protein sources and two Trp:Lys SID ratios

Parameters	Feeding manner		P-Value	Trp:Lys SID Ratio		P-Value	CV
	Vegetal	By-products		18%	21%		
FW, kg	64.13	63.72	0.418	62.97[b]	64.92[a]	0.014	3.64
DFI, kg	2.128[a]	2.052[b]	0.032	2.064	2.118	0.198	5.92
DWG, kg	1.064	1.048	0.433	1.029[b]	1.085[a]	0.014	6.81
FE	0.501	0.522	0.079	0.499[b]	0.513[a]	0.018	4.01

a or b - means followed by different letters in the lines are different by the F test (P <0.05)
FW – final weight.
DFI – daily feed intake.
DWG – daily weight gain.
FE – feed efficiency.

Table 5. Performance of swine from 70 to 95 kg fed with two protein sources and two Trp:Lys SID ratios

Parameters	Feeding manner		P-Value	Trp:Lys SID Ratio		P-Value	CV
	Vegetal	By-products		18%	21%		
FW, kg	92.62	93.24	0.701	91.71[b]	94.20[a]	0.012	3.01
DFI, kg	2.732	2.724	0.815	2.650[b]	2.810[a]	0.003	5.95
DWG, kg	0.983	0.989	0.828	0.946[b]	1.028[a]	0.002	8.17
FE	0.360	0.363	0.538	0.357	0.366	0.133	5.25

a or b - means followed by different letters in the lines are different by the F test (P <0.05)
FW – final weight.
DFI – daily feed intake.
DWG – daily weight gain.
FE – feed efficiency.

The results demonstrate that animal by-products in the swine diet do not negatively affect the final performance. Its use will depend directly on price, quality and availability to the nutritionist.
For the author, the Trp: Lys SID ratio of 18% was sufficient to meet the performance of swine in the period from 15 to 25 kg. On the other hand, swine over 30 kg fed with the Trp:Lys SID ratio of 21% showed improved performance responses. The modulation of the ghrelin hormone (GH) in the stomach of swine, with consequent GH expression, may have caused a better animal performance (Pereira, 2014; Zhang et al., 2007 and Correia-Silva, 2009). Also, the higher content of tryptophan in diets, regardless of the protein sources used in this experiment, may have promoted the more desirable Trp/LNAA ratio in plasma, thus enhancing the absorption of tryptophan in the blood-brain barrier and improving the synthesis of serotonin (Adeola and Ball, 1992), reflecting in the best final performance of the animals.

Conclusion

Protein sources from vegetables (soybean meal) or animal by-products (meat and bone meal + feather meal) can provide the same final performance in swine. Thus, the price of the animal by-products when associated with the quality and availability seems to be the fundamental criteria for use in diets for pigs over 15 kg.
The Trp:Lys SID ratio of 21% in swine over 30 kg, regardless of the protein sources studied, is an excellent option for nutritionists who aim for improvement of performance, optimal health and welfare of their animals.

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Histidine requirements of broilers for protein synthesis and beyond

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INTRODUCTION

Reducing emission across a variety of industries has been the focus for years. Global warming and climate change have shifted part of the focus to the agriculture. Thus, agriculture and specifically animal production is under pressure to reduce nitrogen and phosphorus emissions and reduce gaseous pollutions such as CO2 and methane. Phytase has been used for decades to reduce phosphorus excretion and to increase phytate phosphorus availability to the animals. To reduce the nitrogen emissions, modern monogastric diets have switched from protein optimization to amino acid optimization. Lysine, methionine, threonine, tryptophan, and valine are commonly used in feed formulations. Isoleucine and arginine have been recently introduced to the market as supplementary amino acids. Based on these, the industry is designing low protein and functional diets to contribute in reducing the impact of animal production on environmental pollution. Histidine (His) is the next limiting amino acid which is already registered and used in parts of the world in EU. Herein, the role of His and its requirements in monogastric species is explained.

Histidine requirements in broilers

The His requirement for maximum weight gain and feed efficiency is determined to be not more than 0.31% (8-22 days post hatch) in broiler's diet in which the His digestibility was determined to be 81.4% (Han et al. 1991). The optimal ratios of digestible His (dHis) to digestible Lys (dLys) for starter phase in the literature are 27% (Dean and Scott, 1965), 32% (Huston and Scott, 1968), 36% (Sasse and Baker, 1973), 36% (Baker and Han, 1994), and 36% (Dorigam et al., 2013). Wecke and Liebert (2013) summarized results of 12 experiments and ended up in a His to Lys ratio (His/Lys) of 34±4. The most recent study on His requirement in broilers was conducted by the Dilger research laboratory and published by Franco et al. (2017) at the University of Illinois. On average a His/Lys of 38% was reported and they also observed a higher His requirement for carcass parameters as compared with body weight and FCR in 8 to 17 days old birds (Table 1). Broiler breeder companies (ROSS and COBB) currently do not have His recommendations. Brazilian tables suggest 37% His/Lys for all ages of broilers. Same tables suggest dHis requirements of 0.49, 0.48, 0.46, 0.40, and 0.36% for 1-7, 8-21, 22-33, 34-42, and 43-46 days of age for high performing broilers (Rostagno et al. 2017).

Table 1. Summary of estimated optimal digestible amino acid ratios for broiler chicks
(Adopted from Franco et al., 2017)

		Model[1]						
Amino acid	Response	L	Q	95% Quad	LRP	LRP/Quad	QBL	Mean ratio[2]
Histidine	Body weight gain	40	-	-	-	-	-	
	Feed conversion ratio	-	36	34	31	34	32	
	Breast weight	-	39	37	36	38	40	38
	Breast fillet weight	-	41	39	36	39	40	
	Breast yield	-	41	39	37	39	41	
	Breast fillet yield		39	40	37	38	42	

[1] Optimal digestible ratio estimates shown for each response in which at least one model was significant.
[2] Overall mean estimated optimal digestible ratio across all significant response variables per amino acid.
Abbreviations: L, linear; Q, quadratic; 95% Quad, 95% of the asymptotic parameter of the quadratic model; LRP, linear response plateau; LRP/Quad, LRP-to quadratic regression ratio; QBL, quadratic broken line.

In a typical corn soybean broiler feed diet, His/Lys is around 43% (starter phase) under a normal protein level (21.7% CP). An attempt to reduce the protein content down to 20% would reduce the His/Lys to 38%. Thus, after arginine and isoleucine, histidine would be the next limiting amino acid in corn soybean diets. In a wheat corn soybean diet, His/Lys starts from 40% in a normal protein condition and a similar attempt to reduce crude protein would cause an isoleucine, leucine and glycine plus serine deficiency as well as drop of His/Lys to below 38%. These are some examples showing the importance of His under different formulation conditions.

Physiological roles of histidine

Histidine is an integral component of a broad set of tissues including skin, feather, bone, ligaments, and obviously muscle (NRC, 1994). This amino acid also serves to stimulate the digestive secretion of gastrin, a hormone that activates production of hydrochloric acid and pepsinogen, which are essential for digestion of dietary protein (Berdanier, 1998; D'Mello, 2003).
Histidine together with β-alanine are needed for carnosine synthesis which is a dipeptide synthesized by the carnosine synthase. High concentration of carnosine is found in muscle and brain tissues, especially in breast muscle of mammalian and avian species (Kohen et al., 1988; O'Dowd et al., 1988; Biffo et al., 1990). Recently, carnosine has gained increasing attention as a functional ingredient for human food because of its high antioxidant activity (Mozdzan et al., 2005), high buffering capacity to maintain intracellular pH change (Abe, 2000), and anti-glycating and anti-aldehyde effects (Aldini et al., 2005; Guiotto et al., 2005).
Histidine supplementation in broiler diets increases the concentration of carnosine in breast muscle of 1-32 days old broilers (Park et al., 2013). To increase concentration of carnosine in breast muscle tissue of broilers using L-His or spray dried blood cells (SDBC, a His-rich raw material), only the L-His supplemented diet could increase the concentration of carnosine and improved the antioxidative status of breast muscle (Kopec et al., 2013). Whereas, the spray dried blood cells caused a loss in performance parameters probably because of a very high leucine concentration in SDBC contrary to L-His which improves performance.
Breast muscle has about 3 times higher carnosine concentration than thigh muscle (Barbaresi et al., 2019). Moreover, slow growing chickens presented higher carnosine concentration in breast muscle compared with ROSS 308 (*ad libitum* fed or limited fed) (Barbaresi et al., 2019). Recently, concentration of carnosine is also linked to the incidence of breast muscle diseases (Soglia et al., 2019). Severe cases of breast gaping presented a low concentration carnosine. Thus, there is a potential in His to help the industry solving similar issues. Having in mind that some of the nutritional trends of last decades has been ban of animal protein sources (meat and bone meal and blood meal) and lowering the concentration of protein in the diets both of which has dramatically reduced the concentration of histidine in feed formulations.

Conclusion

Histidine is getting a fresh attention in broiler nutrition as the next limiting amino acid. New nutritional trends had tremendously reduced concentration of His in broiler's diet. CP reduction has urged the need of crystalline His for commercial broiler diets. Histidine is a functional amino acid with antioxidative properties. As described above, it can influence yield and carnosine concentration of broiler's breast filets and thus nutritionists and formulators of broiler diets should balance for His not only to meet nutritional requirements but also to benefit from functional aspects of His as a new solution available on the market.

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L-arginine supplementation in laying hens feed: growth, egg production, egg quality and immunity

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INTRODUCTION

Poultry are unable to synthesize L-arginine (Arg) due to the lack of almost all the enzymes involved in the urea cycle (Khajali and Wideman, 2010). This imperfection in bird's physiology keeps Arg as one of the most crucial and essential amino acids (AAs) in poultry. Therefore, it is important to meet the Arg requirements in the birds through dietary supplementation to achieve the maximum performance.

The constant progress in breeding value of poultry changes the genetic potential of birds. An update about the nutrient requirements of the recent genetics of the laying hens is being ignored. Same is applies to the Arg requirements of the laying hens. There is reasonable amount of literature available about the Arg requirement in broilers, however, there is not enough studies on the requirement of Arg in laying hens. Currently, NRC 1994 recommends 700 mg of Arg/hen/day, Brazilian tables recommends 806 mg/hen/day, Lohmann recommends 830 mg Arg/hen/day at the feed intake of 110g/d, whereas, Hy-Line recommends 856 mg/hen/day.

Laying performance

Increasing Arg in diets seem to have a positive effect on laying rate (Silva et al., 2012). Arg inclusion in diets higher than NRC recommendation caused better results. With Arg inclusion rates at 0, 200, 400 and 600 mg/kg, a dietary digestible Arg level of 0.943%, 1.093%, 1.243%, 1.393%, and 1.543% was achieved. At 1.262% of dArg, the highest egg production was obtained. An increase in dietary Arg by 2% and 4% (0.714% and 0.728%, respectively) above NRC recommendations (0.70%) in diet of layers had positive effects on egg production and egg mass with at least 6% of extra production as compared with the control (based on NRC) (Youssef et al., 2015). Likewise, L-Arg supplementation to broiler breeder hens (60 week of age, 210 Arbor Acres female broiler breeders) by 0, 2000, 4000, 6000, and 8000 mg/kg, contributing to 0.96%, 1.16%, 1.36%, 1.56%, and 1.76% dArg, respectively, resulted in highest laying rate at 1.36% dArg level (Duan et al., 2015).

In a recent study (Leiboldt et al., 2016), different genotype (GT): two white (WLA and R11, high and low performing GTs, respectively) and two brown (BLA and L68, high and low performing GTs, respectively) layers were considered. Authors hypothesized that high performing genotypes (HPGTs) and low performing genotypes (LPGTs) will perform differently with L-Arg level of 70% of NRC as low Arg (LA), 100% as adequate arginine (AA) and 200% as high arginine (HA). GT has effect on laying parameters. The laying intensity did not differ between HPGTs, whereas LPGTs. In general, the deficient Arg group (LA) showed lower laying intensity than AA and HA ($p < 0.05$). Egg weight was influenced by GT, diet and Age as well as their interactions ($p<0.001$). Brown eggs were heavier than white eggs. HPGTs laid heavier eggs than LPGTs until week 33 ($p<0.001$). Daily egg mass (DEM) was affected by GT, diet, age and interaction of GTs and age. In general, deficit group showed lowest DEM among all GTs. Yolk proportion was affected significantly by GT, diet, age and interaction between GT and diet, GT and age. HPGT eggs contained lower yolk proportion than LPGT eggs ($p<0.001$). Egg shell proportion was affected by GT and age also by interaction between GT and diet, GT and Age, GT and diet and age ($p<0.001$). White eggs had higher shell proportion than brown eggs.

In conclusion, high level of Arg have a positive effect on secretion of luteinizing hormone (LH). LH acts directly on the ovary and follicles. Arginine imbalance in this case may cause lower laying rate.

Egg quality

Egg quality parameters such as yolk color, shape index, yolk index, haugh unit, albumin percentage, and shell thickness are mostly not affected by Arg level (Youssef et al., 2015). Lima & Silva (2007) also did not observe any effect of different digestible Arg to Lys ratios on egg quality parameters. Silva et al. (2012) observed a linear increase in egg weight with a higher Arg level although it brought in an opposite effect on specific egg gravity (Basiouni et al., 2006). Yang et al. (2015) supplemented 0, 8.5 and 17.0 mg L-Arg per kg of feed in 25-week-old brown leghorn hens. L-Arg had no negative effects of egg production and egg quality. Yolk color was evaluated with DSM yolk color fan (expressed in 15 grades). Yolk color was increased in group 17.0 mg L-Arg per kg of feed compared to control group. In general, the effects of Arg on egg quality looks limited.

Immunity

L-Arg has direct and indirect influence on immunity. In Leghorn type chickens, 30% L-Arg above NRC recommendations caused an increase in anti-sheep red blood cell (anti-SRBC) antibody level and a reduction in relative bursa weight in high Arg fed birds. Short-term supplementary L-Arg had minimal effects on immunity, but some enhancement of SRBC antibody responses in later stages of growth was observed with previous L-Arg administration (Deng et al., 2005). Supplementing L-Arg in laying hens significantly increased WBC and lymphocyte cells while significantly decreased the percentages of heterophils and the ratio of heterophils to lymphocyte in comparison to the control group (Youssef et al., 2015; Al-Hassani et al., 2011).

In breeder hens, 1.36% dArg significantly elevated total antioxidative capacity of birds and reduced the malondialdehyde (MDA) concentration in all tissues. Higher L-Arg levels was disadvantageous (Duan et al., 2015) showing the important of Arg balance to enhance antioxidant capacity via nonenzymatic and enzymatic antioxidant systems. A decrease in serum MDA content suggest that Arg may protect tissues against lipid oxidation especially in the cell walls.

IgY content in eggs was 62.8% higher and IL-2 was 41.8% higher when 17mg L-Arg per kg of feed was supplemented to birds comparing to control birds not receiving supplemental L-Arg (table 1; Yang et al., 2015).

Table 1. Effect of L-arginine on immune parameters of eggs

(Adopted from Yang et al., 2015)

Items	Arginine (mg/kg)		
	0	8.5	17
IgY content (μg)	90.92^{a}±10.53	104.42^{ab}±8.71	148.02^{b}±35.14
IL-2 (ng/l)	3.85^{a}±0.37	4.31^{ab}±0.38	5.46^{b}±0.69
IFN-y (ng/L)	60.81^{a}±6.49	68±6.86	79.02±9.76

^{a,b} Mean ± SE with different superscripts within a column are significantly different ($p<0.05$)

Conclusion

Based on the literature study, CJ has established the ideal amino acids ratio for the laying hens. The ideal Arg to Lys in pre-lay period is found in the range of 106-115% and for the laying period is 112%. Beneficial effects of Arg beyond performance should be taken into consideration as well. Immune status of layers can be improved by supplementing Arg above the requirements.

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Reducing the crude protein content in broiler diet and its impingement

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Abstract

Protein is a vital nutrient for poultry which plays a significant role in growth, egg production, immunity, adaptation to the environment, and in many other biological functions which are attributed to specific amino acids. Poultry diets with lower crude protein have generated global interest from the industry due to its impact on lowering feed costs, improving feed utilization, reducing environmental impacts, and minimizing health and welfare concerns. This article highlights the literatures regarding the effect of low crude protein in broiler industry and its overall impact on the performance, health, environment and welfare. According to the studies, there is room for further reduction in crude protein level with the balanced amino acid nutrition with the special emphasis on the non-essential amino acids i.e. glycine and branched chain amino acids and with the addition of suitable feed additives. Reducing protein levels can also address to the incidence of foot pad dermatitis and necrotic enteritis in broilers while reducing the nitrogen excretion. The concerned field requires further literatures to enable nutritionists for successful integration of synthetic amino acid with the low protein levels in poultry industry with the proper knowledge on the nutrient requirements, their interactions, its impact on environment and the feasibility to optimize the feed cost.

Background

The concept of reduced protein emerged at global level due to competition for feed ingredients among human and animals leading to rise in the cost, health and welfare concerns among consumers as well as the environmental impact of poultry industry in relation to the nitrogen emission. Agricultural and animal production sectors are major contributors to the nitrogen emission. The shift towards lower protein has been a gradual process of evolution and the history goes back to the early 1990's. The reduction in the crude protein level is feasible because of the availability of synthetic amino acids (AAs) such as methionine (Met), lysine (Lys), threonine (Thr), tryptophan (Trp), valine (Val), arginine (Arg), isoleucine (Ile) and glycine (Gly). Using supplemental AA to meet the requirements to achieve the desired production performance thereby reducing crude protein (CP) is possible today, and as a result, the need for intact protein sources such as soybean meal can be reduced.

This review is a compilation of data on the recent studies conducted on the effect of reduced crude protein diet in broilers and its overall impact. The aim of this document is to gauge the current knowledge on the effects of low CP diets fed in broilers, and to highlight the use of synthetic amino acid in low CP balanced diets. This can be a tool to optimize feed costs while maintaining the production performance of birds and simultaneously reducing the excretion of nitrogen. This adds to the further scope of synthetic amino acid addition in poultry diet. There is still limited use of synthetic AAs due to the limited knowledge regarding the nutrient content of feed ingredients and the specific requirement of a particular species at different age and environmental conditions. Nutritionists come into role to formulate rations more precisely to meet the animal's AA needs, as well as providing a flexibility in raw material selection adding profit to the producers.

Reduced protein in broilers

Despite the large body of scientific literatures available showing the positive effects of low protein diets in broilers, still there is a scope of improvement in this field due to the misconception of decrease in performance with the reduction in protein level among producers. The current literatures (2010-2020) collected in Table 1 depicts the comparison of reduced protein diet supplemented with synthetic amino acids with the standard one in broilers. With the decreasing crude protein level, protein efficiency ratio (PER) linearly increase in poultry diet (Cheng et al., 1997). This leads to lower feed cost in low-protein diet but the drawback to this approach is the strong negative effects on weight gain and feed conversion ratio. Many nutritionists attempted to overcome this problem by fortification with amino acids (AA) during the last 3 decades which may result in a better economic return for the industry and lessen environmental pollution (Pesti, 2009). However, only a narrow margin of CP may be reduced by essential amino acids (EAA) fortification (Awad et al., 2014). The possible reason can be the insufficiency of nitrogen supply for non-essential amino acids

(NEAA) synthesis. As steps are taken to reduce crude protein, it is becoming appropriate to consider the supply of non-essential amino acids that we have taken for granted as being supplied in sufficient quantity with the allegedly surplus protein. Accordingly, several studies have been carried out to investigate the effect of NEAA supplementation in low-CP, EAA-fortified diets (Dean et al., 2006) and revealed that when low-CP diet is supplemented with a mixture of NEAA, it enhanced the growth performance of birds. Again, similar improvement was reported when the low-CP diet was fortified with only glycine in the broiler studies conducted by Dean et al. (2006) and Ospina-Rojas et al. (2014). The importance of considering glycine and serine in low protein diets was discussed by Siegert and Rodehutscord (2015), where the proteins in the chicken body incorporating both glycine and serine and the metabolic processes requiring adequate supplies of these amino acids are highlighted. Nutritionists should again consider the importance of individual amino acids and their role in maintaining gut health when there is restricted use of antibiotic growth promoters. In particular, mucin production is of relevance because it aids in protecting the gut mucosa and is dependent upon adequate glycine and serine levels. Siegert and Rodehutscord (2015) emphasized the importance of interrelationships between essential and non-essential amino acids and the need to consider increasing proportions of, for example, threonine as a precursor for the synthesis of glycine. It is also pointed out that, as commercial nutritionists are tempted to use higher levels of DL-methionine to maintain ideal protein profiles, there is the factor of the role of glycine in the conversion of methionine to cysteine and in diets with low cysteine the requirement for glycine may be increased. The conclusion drawn is that, with dietary protein concentrations below 20%, glycine and serine will become limiting and that a glycine equivalent value will be required for diet formulation as an indicator of future direction for feed formulation. Ospina-Rojas et al. (2013) recommended 2.08% total Gly+Ser in a low-CP diet (19% CP) for male broilers from 0 to 3 weeks of age. Awad et al. (2015) showed that providing a level of 2.03% total Gly+Ser to a 16.2% CP diet failed to support optimal growth performance in male broiler chicks kept under tropical climate. Beside the environmental factor, two more possibilities may explain the inconsistency between results. The first is the possibility of higher Gly + Ser requirements under tropical climate. The high environmental temperature may profoundly affect the AA requirements of broilers (Balnave, 2004) through a reduction in AA digestibility (Soleimani et al., 2010). Secondly the use of male broiler chicks in (Awad et al., 2015) might be the reason for not achieving a standard growth performance. According to Baker (2003), male broilers are estimated to have higher AA requirements than females. In this regard, literature by Hernandez et al. (2012) revealed by adopting a low-CP 4-phase feeding program, adverse effect on the performance of male broilers but not females.

Table 1. Recent studies conducted on effect of reduced CP level on overall performance of broilers

References	Age (Day)	Strain	Diets	BW (g)	Feed Intake (g)	FCR	Nitrogen %
Laudadio et al., 2012	1-42	Hubbard	22.5% CP+ L-Lys + DL-Met + L-Thr	2488	91.5	1.55	
			20.5% CP+ L-Lys+ DL-Met + L-Thr	2502	92.1	1.54	
			18.5% CP+ L-Lys+ DL-Met + L-Thr	2403	89.2	1.59	
Awad et al., 2014	1-21	Cobb 500	16.2% CP+ L-Lys + DL-Met + L-Arg + L-Thr + L-Val + L-Ile + L-Phe + L-Trp + L-Leu + L-His + L-Gly	632^{c}	953^{b}	1.60^{a}	
			17.7% CP + L-Lys + DL-Met + L-Arg + L-Thr + L-Val + L-Ile + L-Trp + L-His	747^{b}	1047^{a}	1.48^{b}	
			19.2% CP + L-Lys + DL-Met + L-Thr + L-Val + L-Ile + L-Trp	805^{a}	1043^{a}	1.37^{c}	
			20.7% CP + L-Lys + DL-Met + L-Thr	816^{a}	1084^{a}	1.39^{bc}	
			22.2% CP + L-Lys + DL-Met + L-Thr	830^{a}	1078^{a}	1.36^{c}	
Awad et al., 2015	1-21	Cobb 500	22.2% CP (PC)	923^{a}	1186^{a}	1.35^{b}	
			16.2% CP + EAA (NC)	730^{c}	1050^{d}	1.54^{a}	
			NC + Gly	844^{b}	1119^{bc}	1.41^{b}	
			NC + Glu	773^{c}	1117^{bc}	1.54^{a}	
			NC + Pro	761^{c}	1075^{cd}	1.51^{a}	
			NC + Ala	758^{c}	1081^{cd}	1.52^{a}	
			NC + Asp	775^{c}	1097^{cd}	1.51^{a}	
			NC + NEAA	902^{a}	1164^{ab}	1.36^{b}	
Awad et al., 2017	1-21d-Starter 22-42d-Grower	Cobb 500	Starter-22.2%, Grower 19.5% (PC)	2251^{a}	4073^{a}	1.81^{b}	3.29^{a}
			Starter-16.2%, Grower 13.5% (NC)	1883^{c}	3607^{c}	1.92^{a}	2.21^{b}
			NC+(Gly+Ser) 2.02 - Starter NC+(Gly+Ser) 1.78 - Grower	2035^{b}	3690^{bc}	1.81^{b}	2.36^{b}
			NC+(Gly+Ser) 2.22 - Starter NC+(Gly+Ser) 1.96 - Grower	2071^{b}	3750^{b}	1.81^{b}	2.41^{b}
			NC+(Gly+Ser) 2.42 - Starter NC+(Gly+Ser) 2.14 - Grower	2091^{b}	3768^{b}	1.80^{b}	2.44^{b}
Belloir et al., 2017	21-35	Ross PM3	19% CP	2460	2430	1.64^{b}	
			18% CP	2470	2477	1.65^{b}	
			17% CP	2466	2472	1.65^{b}	
			16% CP	2451	2459	1.69^{a}	
			15% CP	2461	2528	1.71^{a}	
Belloir et al., 2017	21-35	Ross PM3	19% CP	2288	2242	1.68	
			17.5% CP	2332	2323	1.68	
			16% CP	2324	2348	1.71	

References	Age (Day)	Strain	Diets	BW (g)	Feed Intake (g)	FCR	Nitrogen %
*Ullrich et al., 2018	1-35	Ross 308	CP-Control	2192[bc]		1.848	50.0[a] g/kgDM
			CP-1	2244[ab]		1.817	47.9[a] g/kgDM
			CP-2	2329[a]		1.776	42.4[b] g/kgDM
			CP-3	2131[c]		1.827	31.7[c] g/kgDM
Hillar et al., 2020	21-35	Ross 308	20.0% CP	BWG:1404	2230[a]	1.592	
			18.5% CP	BWG:1325	2121[a]	1.607	
			17.0% CP	BWG:1209	1955[b]	1.609	

Impact on health, welfare, and environment

The source and level of dietary protein affect the intestinal *Campylobacter perfringens* populations with high or excess protein associated with greater incidence of necrotic enteritis or bacterial imbalance leading to enteritis and hence wet litter (Drew et al., 2004). It was estimated that 1% excess protein increases water intake by 3% in broilers by Larbier and Leclercq (1994). Wet litter causes higher incidence of pododermatitis, hence the avoidance of any increase in water use is regarded as essential. Dietary protein content is also correlated with water consumption and excretion (Alleman and Leclercq, 1997), and higher dietary CP levels has shown to increase the incidence of wet litter in poultry. Higher water consumption may result from the sodium dependent AA transporters drawing water across the lumen with greater AA absorption. Wet litter occurs as a result of increased water excretion and water spillage from more frequent visits to the water lines. Wet litter is known to cause dermatological diseases such as foot pad dermatitis and cellulitis. Skin infections are the main causes of carcass and chicken paw downgrade, reducing the yield of the meat chicken industry (US Poultry & Egg Export Council, 2009). Undigested protein that exits the small intestine acts as a substrate for the bacterium *Clostridium perfringens* in the hindgut, a pathogenic bacterium responsible for necrotic enteritis (Drew et al., 2004). The higher N waste levels (Ferguson et al., 1998), odorants (Sharma et al., 2017) and wet litter (Wheeler and James, 1949) that are associated with higher CP diet, creates an optimal environment for disease and infection. With the decreasing level of protein, these effects are reduced (Belloir et al., 2017), risk of diseases are lowered and animal welfare is improved. With the restriction of antibiotic growth promoters in poultry diets, the risk of necrotic enteritis and other related intestinal diseases will increase where any disease preventative measures, including reduction of dietary CP hold importance.

Excess CP can overload the gastrointestinal tract (GIT) with excessive AA, peptides and undigested protein (Apajalahti and Vienola, 2016). This can hamper feed efficiency, contributes to health and welfare issues and adds to negative environmental impacts. Excess AA presented in the diet are absorbed and catabolized, producing higher levels of N excretion in the form of uric acid (Wu, 2013). Nitrogen wastes have become a focus of environmental sustainability due to their impact on waterway pollution and ecosystems. Reducing dietary CP further promotes the sustainability and marketability of the poultry industry. Improving industry sustainability with low protein (LP) diets comes from reduced water intake and N excretion (Belloir et al., 2017). Decreasing industry dependence on dietary CP improves the health and welfare of meat type chickens by improving living conditions as well as feed utilization. The health benefits of LP diets must also be considered with future regulations on antibiotic growth promoter use. To remain an efficient and sustainable production system, the industry must consider concepts such as LP diets. Due to the direct relationship between dietary protein level and N excretion, the logical solution to excessive excreta N is to reduce protein content in the diet (Lopez and Leeson, 1995).

Conclusion

With a better understanding of specific nutrient requirements along with the possible interactions and other influencing factors, addition of adequate amino acids make it possible to drastically reduce dietary crude protein (CP) in poultry without compromising the profit level. In addition, the increasing availability of feed grade amino acids (i.e. L-tryptophan, L-valine, L-arginine, L-isoleucine, and L-histidine) allow the further decrease of dietary CP and changed the way of addressing risk management in feeds for monogastric animals which can further address to the successful adoption of antibiotic free animal production and thus reducing predisposing factors to disease. However, extensive studies regarding the successful inclusion of low protein diets is required to make their extension practical, profitable and worthwhile for the global poultry industry. The extension of LP diets in the poultry industry promotes a decrease in feed costs, environmental, health and welfare issues and an increase in N utilization. These benefits will result in an improvement in environmental sustainability and marketability in the industry.

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How to use meta-analysis to estimate amino acid requirements in animal nutrition

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CJ BIO, Head quarter

Abstract

In animal nutrition, the need for determining accurate and reliable requirements is increasing to maximize animal growth and performance. A considerable amount of research has been done on amino acid requirements. However, each has a different result and it is necessary to find a single representative value describing the overall patterns of the studies. The purpose of this article is to present a method of meta-analysis used to combine the results from multiple studies.

Background

The excessive or insufficient intake of amino acid has disadvantages such as growth depression and unnecessary cost increase. For these reasons, measuring accurate and reliable requirements are crucial.
For estimating requirements, linear-plateau and curvilinear-plateau models are widely applied (Fig. 1). The basic design of a dose-response trial consist of a basal diet which is deficient in the test amino acid and a graded supplementation of the amino acid. As the amount of amino acid increases, the response (i.e. average daily gain) will gradually increase to the plateau value, at which the growth is maximized. The amino acid intake above this amount would no longer influence the growth performance and will remain constant or decrease again when reaching a critical amount depending on the amino acids. Researchers can then determine the requirement by estimating minimum supply of amino acid to reach the plateau value.
However, the requirement will typically vary across studies. Therefore, it is necessary to integrate quantitative results from multiple studies to find a single representative value. The purpose of this article is to present a method which can be used to solve this problem.

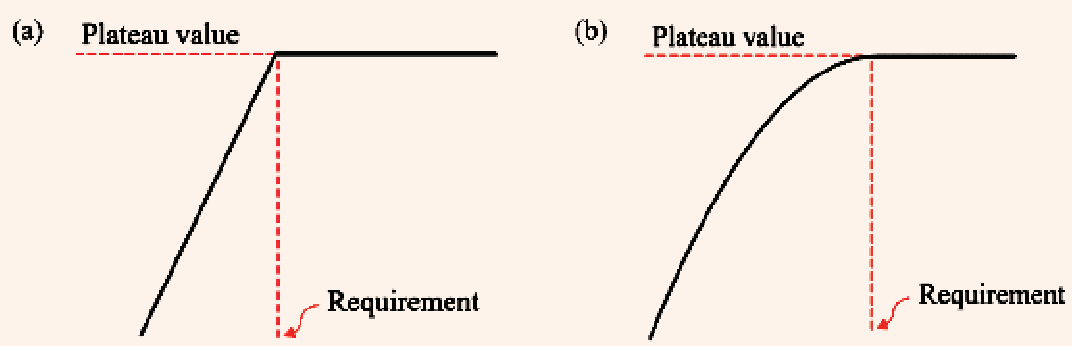

Figure 1. The linear-plateau (a) and curvilinear-plateau (b) models with their plateau values.

1) What is meta-analysis?

Meta-analysis is a statistcal analysis that combines the results from multiple studies. It can be performed when there are multiple studies conducted for the same purpose and with comparable methods. The results then are summarized by a single statistical model representing overall patterns that best explains the observations. By incorporating the results, researchers can obtain more accurate information from meta-analysis compared to a single study.

2) Materials and methods

Data collection

Generally, the data gathered across studies are unbalanced since the result obtained from a single study is specific to the experimental condition. Therefore, to ensure that a reasonable analysis could be carried out, the published studies should be evaluated based on predetermined inclusion and exclusion criteria. A database should then consist of studies with the same selection criteria such as publication year, experimental design, number of amino acid levels and composition of feedstuffs used in experimental diets.

Statistical models

Once the studies are collected, the linear-plateau (LP) and curvilinear-plateau (CLP) models are applied to the data. These models can be written as follows:

Linear plateau:

$$Y_{ij} = \begin{cases} A_i\left(1 + U\left(R - x_{ij}\right)\right) + \varepsilon_{ij}, & x_{ij} < R \\ A_i + \varepsilon_{ij}, & x_{ij} > R \end{cases}$$

Curvilinear plateau:

$$Y_{ij} = \begin{cases} A_i\left(1 + U\left(R - x_{ij}\right)^2\right) + \varepsilon_{ij}, & x_{ij} < R \\ A_i + \varepsilon_{ij}, & x_{ij} > R \end{cases}$$

where Y_{ij} is the growth performance, X_{ij} is the amino acid supply, A_i is the plateau value (i.e. maximum growth), R is the minimum amino acid value which maximizes the growth and U is a parameter to be estimated. The choice of the statistical model depends on the shape of the data. Figure 1a indicates that the rate of change between the two variables, growth and amino acid, is constant while Figure 1b indicates that it is not constant. Therefore, the type of statistical regression model used to determine the requirement may result in different dietary recommendations. Among the two different types of models, a more appropriate model in the context can be determined using Akaike Information Criteria (AIC); the model that minimizes AIC is considered more suitable. Therefore, it is possible to determine the requirement by comparing AIC of the LP model with that of CLP. Statistical analysis can be conducted using R or SAS procedures.

3) Example: Estimation of lysine requirement in broilers

To determine digestible lysine requirement in broilers, a database was constructed using 8 experiments based on the following selection criteria: reported after 2000, concerned broilers from 1 to 10 days and aimed to estimate digestible lysine requirement with at least three different levels of lysine. The linear-plateau (LP) and curvilinear-plateau (CLP) models were fitted to estimate lysine requirement using average daily gain as response criteria.
The estimated lysine requirements were 1.22% using the LP model and 1.37% using the CLP model (Fig. 2). The AIC obtained from these two models were 185.1 and 181.2, respectively. The AIC of the CLP model was slightly lower than that obtained from the LP model, suggesting that the CLP model fits better. Therefore, the digestible lysine can be proposed to be 1.37% according to the CLP model.

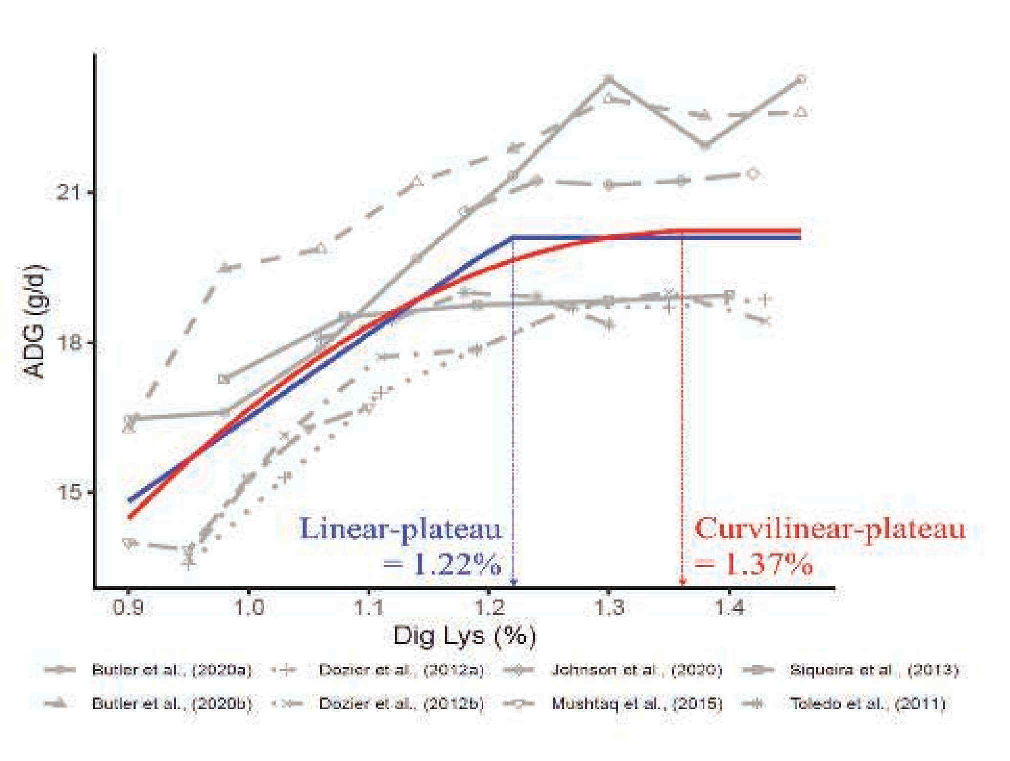

Figure 2. Fitted linear-plateau and curvilinear-plateau models of average daily gain (ADG) as a function of digestible lysine.

Conclusion

Identifying accurate and reliable requirements of amino acid is essential to maximize growth performance and profitability. It is necessary to perform meta-analysis to extract quantitative results among studies since the results vary depending on the study conditions. We can estimate more accurate requirements using meta-analysis and may suggest to update the previous requirements.

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