Factors influencing the study of Tryptophan requirement in pigs

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Abstract: Due to many influencing factors, there is a large discrepancy among estimates of Tryptophan (Trp) requirement of pigs. This article provides theoretical basis for the application of Trp in practice by discussing the factors influencing the study of Trp requirement of pigs including analyzing methods, research methodologies, assessment indices, nutrient level in diets, breeds and growth phase of pigs, environment and health status etc.

Key words: Tryptophan requirement influencing factors

Tryptophan (Trp) is one of the essential amino acids of pigs. In recent years, as more researches have been conducted to study the theories of balanced amino acids nutrition, more attention has been paid on the Trp nutrition. As the conception of ideal protein pattern has been announced, many researchers (Fuller, et.al., 1989; Chung and Baker, 1992) have developed ideal amino acids profile of pigs and ARC (1981) and NRC (1998) have also suggested their ideal amino acids profile respectively. However, there is a large discrepancy of Trp requirement among these profiles. For example, the Trp requirement for weanling pigs ranges from 0.12-0.21% (ARC, 1981; NRC, 1998) and the requirement for finishing pigs ranges from 0.06-0.17% (ARC, 1981; Burgoon et.al., 1992; Zhang et.al., 2001). This mainly due to the fact that the intra-organic metabolism and trophism of Trp is more complicated than other amino acids. Trp can be transferred into 5-Hydroxytryptamine and Nicotinic acid (Han et.al., 1993). Meanwhile, when Trp is entering the brain via blood brain barrier and being transferred into 5-Hydroxytryptamine, there is an interaction between Trp and other large neutral amino acids (LNAA) such as leucine, isoleucine, valine, phenylalanine and tyrosine resulting in a lot of uncertain factors in the study of Trp. The discrepancy of Trp requirement in different researches has brought commercial formulators with a lot of trouble in the application of Trp in practice and has restricted the development of ideal amino acids profile. Therefore, it's important and practical to understand the precise Trp requirement.

There are many factors affecting the requirement of Trp, such as breeds, growth phase, growth performance, diet composition, energy and protein level, amino acids digestibility and availability and assessment indices etc. It's difficult to analyze Trp precisely and there's no uniform analyzing standard around the world. Furthermore, the analytical error of Trp analysis should not be neglected. This article provides theoretical basis for the application of Trp in practice by discussing the factors influencing the study of Trp requirement of pigs including analyzing methods, research methodologies, assessment indices, nutrient level in diets, breeds and growth phase of pigs, environment and health status etc.

1 The accuracy of the analyzing methods of Trp

There are many factors influencing the analysis of Trp. Factors such as hydrolysis methods and hydrolysis degree, sample collection, solvent variation, dissolved oxygen, reagent impurity, solvent container, filters, individual operation variation and subjectivity in baseline selection etc can affect the accuracy of the analysis (Li, 2002). Baseline selection, hydrolysis methods and hydrolysis degree are considered as the main factors affecting the accuracy. The coefficient of variation of results of amino acids analysis especially Trp analysis is large between individuals from the same lab or different labs. Trp has an indole group which makes the analysis more difficult and the analytical error more varied. It's normal if the coefficient of variation varies within 10-20%.

Nowadays, the main analyzing methods of amino acids are high performance liquid chromatography (HPLC) and ion exchange chromatography. Ion exchange chromatography makes resin not easy to be contaminated and has a good separation effect. Moreover, it's easy to clean when using ion exchange chromatography and the resin can be used repeatedly; while the column of HPLC can not be used repeatedly and the results are easy to be influenced by fat and nonpolar substance. Therefore, ion exchange chromatography has a great accuracy and is considered as the standard official analyzing method, but due to its simple use, HPLC has been widely used for experimental determination. Moreover, experimental operation or reagent can cause errors in Trp analysis. Therefore, it's advisable to use official standard method in order to minimize the analytical errors.

In recent years, alkaline hydrolysis has been widely used in hydrolysis method of Trp. Sodium hydroxide is initially used for hydrolysis (110℃, 20h, Nielsen and Hurrell, 1985), but sodium hydroxide is caustic to glass and 97-99% of hydrolyzed Trp can be recovered; so many researches have been conducted to find alternative alkaline solution. Slump et.al., (1991) have compared the effect of Trp hydrolysis by using lithium hydroxide, barium hydroxide and sodium hydroxide and suggested that hydrolysis effect was the best when using 4M barium hydroxide (110℃, 20h). The similar results have been reported by Delhaye and Landry (1993). Therefore, barium hydroxide has been used the most for Trp hydrolysis.

2 Research methodologies

Nowadays, the study of Trp requirement has been conducted combined with ideal amino acids profile. Research methodologies include: (1) Analysis of correlation between input-output. Requirement and the profile can be deduced from the growth performance (Chung and Baker, 1992; Baker and Han, 1994), but this method has certain blindness and randomicity. (2) Amino acids composition analysis (Fuller et.al., 1993). This method focuses on results from digestibility, but does not take into account the effect of difference of amino acids turnover rate during maintenance and N retention and thus may over- or under-estimate the requirement of certain amino acids. (3) Summary of results from the studies of amino acids requirement. Due to the difference of research condition, it's hard to avoid the deviation between ideal amino acids profiles obtained from the literature review and the profile in real world (NRC, 1998). (4) N balance deducted from specific amino acids (Wang and Fuller, 1989; Fuller et.al., 1989). Although the mathematic model used in this method has to be improved further, researches (Chung and Baker, 1992) have indicated that the ideal amino acids profile of growing pigs developed using this method has a high accuracy and therefore results suggested by Wang and Fuller (1989) and Fuller et.al., (1994) have become one of the important foundations for the ideal amino acids profile developed by NRC

(1998). It can be concluded that using different methods to study the ideal amino acids profile can result in the difference in results and thus the discrepancy of Trp requirement in pigs among studies.

3 Assessment indices

Main indices to determine Trp requirement include daily weight gain, feed conversion and serum urea nitrogen etc. Results are varied when using different assessment indices to study Trp requirement. Researches by Burgoon et.al., Wu et.al., (1994) and Lin et.al., (1999, 2001) have suggested the curvilinear relationship between feed intake, daily weight gain and feed:gain ratio and dietary Trp level. It indicates that before the requirement has been met, there is a significant relationship between the growth performance of growing and finishing pigs and dietary Trp level; while the requirement has been met, there is no benefit to increase dietary Trp level. It has been reported by Han et.al., (1993) that the maximum daily weight gain was achieved when digestible Trp level reached to 0.137% , while maximum feed conversion was achieved when digestible Trp reached to 0.122%. In other words, after the maximum feed conversion has been obtained, the feed intake may still increase when increasing dietary Trp level. Therefore, Trp requirement to achieve the maximum feed intake is more than the amount to achieve the maximum feed conversion. Research by Gao (2005) indicated that when feed intake has been adjusted by Trp to meet the requirement for animal's growth, as dietary Trp level was increased, the increase of feed intake by each Trp unit decreased and would not stop decreasing till the restrain of feed intake occurred.

Serum urea nitrogen is an index to indicate animal's nutrition level and protein metabolism. It has been reported by Sawadogo et.al., (1997) that when dietary Trp level was reduced from 0.14% to 0.26%, serum urea nitrogen level was decreased linearly and protein and Trp retention rate was increased significantly. Wu et.al., (1994) and Lin et.al., (1999) have also suggested a negative correlation between serum urea nitrogen level and dietary Trp level. Researches showed that Trp level in diets of growing and finishing pigs had similar impact on daily weight gain and serum urea nitrogen (Lin et.al., 2002). Wu (2003) also reported the similar result. Therefore, when serum urea nitrogen is used as assessment index, the Trp level to achieve the maximum daily weight gain can be obtained when serum urea nitrogen level reaches the lowest. However, the amount of Trp to achieve the maximum feed conversion is less, so Trp requirement may be different when using different assessment indices.

4 Nutrient levels

The discrepancy of Trp requirement mainly attributes to the variation of dietary amino acids digestibility, especially when using different diet or synthesized amino acids (Han et.al., 1993). Researches using corn-fish meal-corn gluten meal showed that the maximum growth performance was achieved when total Trp level was 0.205% (Burgoon et.al., 1992, Lin et.al., 2002, Wu et.al., 2003). However, it has been reported by Guzik et.al., (2002) using corn-pea diet that the maximum growth performance was achieved when digestible Trp was 0.18% (total Trp was 0.22%). Due to the fact that the difference in diet may result in the difference in total Trp requirement, it's advisable, in theory, to use digestible amino acids system to study Trp requirement in order to reduce the impact of discrepancy of availability.

4.1 Protein level and Large neutral amino acids (LNAA)

Two main factors influencing the feed intake of pigs are dietary protein and Trp level. Trp requirement can be met by increasing the intake of low protein diet

with low Trp level (Henry et.al., 1992). It has been reported that Trp requirement was increased from 0.071% to 0.119% when protein level was increased from 10% to 18% for 10 kg pigs fed corn-gelatin diet. There is a competition between Trp and LNAA (including isoleucine, leucine, valine, phenylalanine, tyrosine) when entering blood brain barrier. There is a direct relationship between the level of Trp which has crossed the blood brain barrier and the level of neurotransmitter (5-Hydroxytryptamine, dopamine and its metabolites). Therefore, the increase of dietary LNAA level may have impact on animal's feed intake and increase Trp requirement (Henry et.al., 1992). Lin et.al., (2002) has reported that when dietary Trp level was varied in a certain range, as dietary Trp/LLNA ratio was increased, the feed intake of both growing and finishing pigs were increased significantly. When Trp level has met the requirement for maximum growth rate, further increase of dietary Trp/LNAA ratio would result in the decrease of weight gain achieved by each Trp unit.

4.2 Lysine level

It's important to determine dietary lysine level to study the ideal amino acids profile. Researches have shown that based on different protein level or diet, the order of limited amino acids and ideal amino acids profile of pigs are different. Different lysine level can result in the change of Trp requirement. Lin et.al., (2002) have conducted experiments to determine the true digestible Trp requirement (TTrp) of finishing pigs. The results suggested that when total lysine level of growing pigs was 0.75% (true digestible lysine level is 0.685%), TTrp was 0.144%; when total lysine level of finishing pigs was 0.60% (true digestible lysine level is 0.541%), TTrp was 0.114%. These results are lower than the data reported by Zhang et.al., (2001) in which the true digestible lysine level was 0.896% and TTrp was 0.17% when the maximum growth performance was achieved in 20-35 kg growing pigs.

4.3 Nicotinic acid level

When dietary Trp can't meet the requirement of piglets, increasing nicotinic acid level (120 mg/kg) can improve the utilization of protein by piglets (Markant et.al., 1993). However, excessive nicotinic acid has no impact on Trp requirement, but deficient nicotinic acid may increase Trp requirement (Wu et.al., 1994).

5 Growth phase and breed

For piglets and growing pigs, amino acids requirement includes requirement for maintenance and growth. It has been reported that animal's age and weight can affect its maintenance and growth pattern. Amino acids requirement for protein accretion accounts for 90% of total amino acids requirement in 10 kg piglets, but as body weight increases, the percentage increases (Fuller et.al., 1989; Chung and Baker, 1992). Therefore, as the age and the body weight increases, amino acids requirement for maintenance is increased and thus its ratio in amino acids profile is increased correspondingly (Guzik et.al., 2002).

Amino acids profile of lean type pig is different from the one of fat type pig. Growth rate and N accretion is faster for lean type pig and amino acids for protein accretion accounts for larger percentage and thus the requirement for maintenance is relatively low. Because the ratio of Threonine and Trp for maintenance is large, their ratio in ideal amino acids profile for lean type pig is relatively low. Zhang et.al., (2001) have studied digestible lysine, methionine+cystine, threonine, Tryptophan balance pattern of growing and finishing pigs of different genotype. It has been found that lysine:sulfur amino acid:threonine:Trp ratio of Large White × Landrace pig was 100:49:72:19 and the ratio of Yanan pig was

100:78:76:21.

6 Environment and Disease

The immune stimulation by pathogenic and non-pathogenic immunogen towards animals is common in modern and intensive production system and makes animals in sub-health status. It has been reported that the ideal amino acids profile of pigs in normal and stress condition were different. N apparent biological value in Trp deletion group was considerably decreased from the number equivalent to 90.4% of positive control group to 79.4% and the decrease was related to the fact that Trp was the second limited amino acids to synthesize acute phase protein by muscle protein in immune stimulation condition (Li, 2002). Melchior (2004) has reported that plasma Trp level of pigs got chronic pneumonia in stress model was decreased indicating that Trp requirement was increased when pigs were in inflammation and immune response condition.

Factors affecting the study of Trp requirement have attracted more attention from researchers and commercial feed formulator. A lot of impacts induced by influencing factors are becoming avoided. For example, the accuracy of analyzing methods can be guaranteed by applying universal analysis standard. As studies of protein nutrition have been developed further, analyzing methods of Trp, balanced ratio of Trp and other amino acids and Trp metabolism are becoming hot topics in protein nutrition. The application of the ideal amino acids profile in low protein diet can facilitate the development of the study of Trp nutrition and the improvement of swine ideal amino acids profile, and therefore provide reliable theoretical basis for the proper use of Trp.

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