Effects of dietary true digestible Threonine levels on the growth performance, blood indices and immune functions of weaning piglets

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Abstract: This experiment has been conducted to study effects of different true digestible Threonine (Thr) levels on the growth performance, blood indices and immune functions of weaning piglets weighing 10-25 kg which were fed diets formulated based on true ileal digestibility of Thr measured by previous experiment. 90 barrows (Large White × Landrace) with the age of 28±2d and weight of 9.85±0.78 kg were used in this experiment and were allotted into 5 treatments, 6 replicates per treatment and 3 piglets per replicate. Piglets were fed diets with 5 different levels of true digestible Thr at 5.3, 5.8, 6.5, 7.5, 8.5 g/kg respectively. The duration of the experiment is 28 days. On Day 14, all piglets were administered intramuscular injection with albumin egg at 1 mg/kg BW. The concentration of serum anti albumin egg IgG was measured on Day 14, 21 and 28. The concentration of serum free amino acids and serum urea N were measured on Day 14 and 28. Results showed that in the whole experimental period, as the intake of true digestible Thr was increased, the daily weight gain (P = 0.047) and the feed conversion (P = 0.002) were improved, but the average daily feed intake was not affected (P = 0.822). When the intake of true digestible Thr was 5.9 g/d, the optimal daily weight gain and feed conversion were achieved. As the intake of dietary true digestible Thr was increased from 4.1 to 5.0 g/d, serum urea N was decreased (P = 0.009); while the serum urea N was increased (P = 0.001) as the intake was increased from 5.0 to 6.6 g/d. When the intake of dietary true digestible Thr was increased from 4.1 to 6.6 g/d, the concentration of serum free amino acids was increased (P = 0.001), but the concentration of serum free Histidine (His), Isoleucine (Ile) and Valine (Val) were decreased (P = 0.002). As the intake of dietary true digestible Thr was increased, the concentration of serum anti albumin egg IgG was increased and the highest concentration was achieved when the intake was 6.6 g/d. It can be concluded that the requirement of dietary true digestible Thr of 10-25 kg weaning piglets to achieve the optimal immune functions is higher than the requirement to achieve the optimal daily weight gain and feed conversion.

Keywords: true digestible Threonine growth performance immune functions serum urea nitrogen serum free amino acids weaning piglets

1 Introduction

Threonine (Thr) is considered as the second or third limited amino acid of pigs fed corn basal diets (Lewis, 2001). There is little literature about the requirement of true digestible Thr of pigs. Most existing data which are being used are estimated from the NRC model (1998), but little is from the real experiments.

Thr is the main composition of γ- globulin of poultry, rabbits and the human beings (Tenenhouse & Deutsch, 1966). Research by Bhargava et.al., (1971) has suggested that as the dietary Thr level was increased, the antibody titer of the broiler chicken was increased. Researches on pigs have shown that the decrease of sow's plasma IgG concentration can be slowed down by feeding diets with Thr supplemented (Cuaron et.al., 1984). Furthermore, Li et.al., (1999) has reported the relationship between dietary Thr level and the production of antibody of growing pigs and the IgG level. On the whole, the relationship between Thr and the immunity has been reported from all these researches. Therefore, this experiment was conducted to study effects of different true digestible Thr levels on the growth performance, blood indices and immune functions of weaning piglets based on amino acids' true ileal digestibility measured in previous experiment[1].

2 Materials and Methods

2.1 Experimental animals and diets

90 weaning barrows (Large White × Landrace) with the age of 28±2d and weight of 9.85±0.78 kg were used in this experiment and were allotted into 5 treatments, 6 replicates per treatment and 3 piglets per replicate. 3-day adaptation was carried out before the experiment to make sure all experimental piglets can adapt the mash feeds completely. The formal experimental period is 28 days. The basal diets were formulated based on the true ileal digestibility of amino acids in corn, peanut meal and whey powder measured in previous experiment. Synthesized L-Thr (feed-grade, 98.5% purity, purchased from Degussa) was supplemented into the experimental diets at the level of 0, 0.5, 1.2, 2.2 and 3.2 g/kg respectively. The true digestibility of synthesized L-Thr was calculated as 100% (Saldana et.al., 1994) and therefore the concentration of true digestible Thr was 5.3, 5.8, 6.5, 7.5 and 8.5 g/kg in each experimental diet respectively. Other nutrients in diets were formulated to meet or higher than NRC (1998) recommendations. The diet composition and nutrient levels are shown in Table 1.

2.2 Experimental design

Randomized grouping design has been applied in this experiment. Piglets from 5 treatments were fed 5 experimental diets respectively. On Day 14, all piglets were administered intramuscular injection of albumin egg at 1 mg/kg BW. 3 ml blood sample from each piglet was collected from the superior vena cava using anti-coagulation evacuated blood tube (Serum Clot Activator, Greiner Bio-one) after 7 (Day 21 in experimental period) and 14 days (Day 28 in experimental period) of the injection respectively. Following 5-min centrifuged at 3,500×g (800 Centrifuge, Shanghai), serum was separated from the blood sample and stored by cryopreservation at -20℃ for later use. Body weight was measured once every two weeks and feed intake was recorded.

2.3 Feeding management

Feeding experiment was carried out in Nin He breeding pig farm in Tianjin. Experimental piglets were kept in closed house where the room temperature and the air flux were controlled automatically. The room temperature was kept at 28℃ before the experiment and then decreased by 1.5℃ every week until the temperature was maintained at 22℃. The pen was 1.2×1.2 m^2 size equipped with powder coating slotted floor, adjustable stainless steel trough and nipple watering. Piglets were fed mash feeds and were free to get access to feed and water. Deworming and immune programs were carried out according to the routine management.

[1]Wang, X., S.Y. Qiao, M. Liu, and J.R. Wang. 2007. Determination of apparent and true ileal digestibility of amino acids in normally used raw materials of pigs. Chinese Journal of Animal Science 73 (1): 50-53.

Table 1 Diet composition and nutrient levels

	True digestible Thr level				
	5.3	5.8	6.5	7.5	8.5
Diet composition					
Corn	474.6	474.6	474.6	474.6	474.6
Peanut meal	340.0	340.0	340.0	340.0	340.0
Whey powder	100.0	100.0	100.0	100.0	100.0
Corn starch	7.5	7.0	6.3	5.3	4.3
Soybean oil	30.0	30.0	30.0	30.0	30.0
Limestone	5.6	5.6	5.6	5.6	5.6
Calcium dihydrogen phosphate	21.0	21.0	21.0	21.0	21.0
Salt	2.0	2.0	2.0	2.0	2.0
Premix of minerals[a]	5.0	5.0	5.0	5.0	5.0
Premix of vitamins[b]	5.0	5.0	5.0	5.0	5.0
L-Lysine (78%)	6.6	6.6	6.6	6.6	6.6
L-Threonine (98.5%)	0.0	0.5	1.2	2.2	3.2
DL-Methionine (99%)	2.1	2.1	2.1	2.1	2.1
L-Tryptophan (99%)	0.6	0.6	0.6	0.6	0.6
Chemical composition (g/kg)					
Crude Protein (N × 6.25)[c]	206.0	208.0	204.0	207.0	209.0
Lysine	12.1	11.8	11.7	11.6	11.8
True digestible Lysine	10.9	10. 9	10.9	10.9	10.9
Threonine	5.8	6. 4	7.1	8.0	8.9
True digestible Threonine	5.3	5.8	6.5	7.5	8.5
True digestible Met + Cys	7.0	7.0	7.0	7.0	7.0
rue digestible Tryptophan	2.2	2.2	2.2	2.2	2.2

[a] Provided per kilogram of feed: Mn, 100 mg; Fe, 100 mg; Zn, 100 mg; Cu, 150 mg; I, 0.48 mg; Se, 0.3 mg;

[b] Provided per kilogram of feed: VA, 5,512 IU; VD_3, 2,200 IU; VE, 64 mg; VK_3, 2.2 mg; Nicotinic acid, 50 mg; VB_{12}, 27.6 ug; VB_1, 5.5 mg; VB_2, 4.0 mg; VB_6, 3.0 mg; Pantothenic acid, 13.8 mg; Choline Chloride, 551 mg.

[c] Real measured value.

Table 2 Growth performance and the concentration of serum urea nitrogen in weaning piglets

	True digestible Thr level					SEM[1]	P-value	
	5.3	5.8	6.5	7.5	8.5		Linear	Quadratic
Day 0-14								
Daily weight gain	362[b]	384[ab]	398[ab]	454[a]	410[ab]	25	0.048	0.094
Daily feed intake	617	593	596	636	590	33	0.921	0.994
True digestible Thr intake	3.3[b]	3.4[b]	3.9[b]	4.8[a]	5.0[a]	0.22	0.001	0.001
Feed conversion	1.73[a]	1.55[b]	1.50[bc]	1.40[c]	1.45[bc]	0.04	0.001	0.001
Serum urea N (mg/dL)	18.8[a]	13.0[b]	14.7[ab]	16.2[ab]	13.2[b]	1.33	0.013	0.024
Day 14-28								
Daily weight gain	509	575	568	576	561	28	0.234	0.181
Daily feed intake	906	914	922	937	925	46	0.660	0.991
True digestible Thr intake	4.8[b]	5.3[bc]	6.0[b]	7.0[a]	7.9[a]	0.31	0.001	0.001
Feed conversion	1.79[a]	1.59[b]	1.62[b]	1.62[b]	1.65[b]	0.04	0.101	0.030
Serum urea N (mg/dL)	15.9[a]	12.5[ab]	8.7[b]	12.9[ab]	9.1[b]	1.33	0.009	0.015
Whole period								
Daily weight gain	441	476	488	516	491	22	0.047	0.064
Daily feed intake	773	754	768	782	770	35	0.822	0.979
True digestible Thr intake	4.1[b]	4.4[bc]	5.0[b]	5.9[a]	6.6[a]	0.24	0.001	0.001
Feed conversion	1.76[a]	1.58[b]	1.58[b]	1.52[b]	1.57[b]	0.04	0.002	0.001

[a,b,c] The difference between values in the same row with the same superscript or without superscript is considered as not significant ($P>0.05$).

[1] SEM: The standard error of the mean.

Table 3 Serum free essential amino acids content

	True digestible Thr level (g/kg)					SEM[1]	*P*-value	
	5.3	5.8	6.5	7.5	8.5		Linear	Quadratic
Day 14								
Arginine	4.9	4.6	3.6	4.2	4.6	0.44	0.474	0.193
Histidine	1.5^{a}	1.2^{b}	0.9^{c}	1.1^{bc}	0.9^{c}	0.08	0.001	0.001
Isoleucine	1.4^{a}	1.2^{ab}	0.6^{c}	1.0^{b}	0.7^{c}	0.10	0.001	0.001
Leucine	2.6	2.4	2.1	2.6	2.2	0.19	0.363	0.610
Lysine	4.8	4.1	3.9	4.1	5.4	0.48	0.520	0.060
Methionine	1.0^{b}	0.9^{b}	0.9^{b}	1.0^{b}	1.6^{a}	0.10	0.007	0.001
Phenylalanine	2.5	2.4	2.2	2.3	2.3	0.21	0.524	0.635
Threonine	4.7^{c}	5.0^{c}	5.8^{bc}	8.9^{b}	12.8^{a}	1.10	0.001	0.001
Valine	3.2^{a}	2.5^{b}	1.8^{cd}	2.4^{bc}	1.7^{d}	0.19	0.001	0.001
Day 28								
Arginine	5.0	5.8	5.0	5.1	4.3	0.39	0.105	0.078
Histidine	1.7^{a}	1.2^{b}	0.9^{b}	1.1^{b}	1.0^{b}	0.09	0.001	0.001
Isoleucine	1.3^{a}	1.2^{a}	0.7^{b}	0.8^{b}	0.8^{b}	0.10	0.001	0.001
Leucine	2.3	2.7	2.4	2.6	2.4	0.15	0.740	0.479
Lysine	4.9	4.6	5.0	4.7	3.9	0.36	0.104	0.129
Methionine	1.0^{b}	0.9^{b}	1.2^{a}	1.2^{a}	1.0^{b}	0.06	0.310	0.021
Phenylalanine	2.4	2.6	2.6	2.4	2.5	0.14	0.778	0.669
Threonine	3.9^{c}	4.8^{c}	6.5^{b}	8.9^{a}	8.7^{a}	0.54	0.001	0.001
Valine	3.2^{a}	2.9^{ab}	2.1^{c}	2.6^{bc}	2.5^{bc}	0.23	0.016	0.008

[a,b,c] The difference between values in the same row with the same superscript or without superscript is considered as not significant ($P>0.05$).

[1] SEM: The standard error of the mean.

2.4 Analysis indices and analysis methods

2.4.1 Raw materials and diet composition

The crude protein was analyzed according to the national standard method (GB/T 6432-94, 1996) using Semi-automatic Nitrogen Analyzer (Tecator, Kjeltac System 1002). The composition of amino acids was determined using Automatic Amino Acid Analyzer (HITACH L-8800, Japan).

2.4.2 Serum indices

The concentration of serum urea N was analyzed according to the method recommended by Tao et.al., (1982) using Biochemical Analyzer (Bayer, Manufactured Bayer Diagnostics Manufacturing Ltd., Dublin, Ireland). After the protein deposition of 1 ml serum treated by 6% salicylic acid solution, serum urea N was determined using High Performance Liquid Chromatograph (HITACH L-8800 Amino Acid Analyzer, Japan) in which o-phthalaldehyde was used as the analysis reagent. The content of IgG was determined using swine ELISA kit (Bethyl Laboratories, Inc., USA).

2.5 Statistical analysis

Variance analysis was carried out using GLM model in SAS 9.0. Effects of the intake of true digestible Thr were analyzed by multiple comparisons (linear, quadratic). "Replicate" ("Pen") was used as statistical unit when analyzing the effects on the growth performance and blood indices (SAS, 2002). The difference was considered as significant when $P<0.05$.

3 Results

3.1 Effects of the intake of true digestible Thr on the growth performance and the concentration of serum urea N in weaning piglets

Results are shown in Table 2. In the whole experimental period, as the intake of true digestible Thr was increased from 4.1 to 6.6 g/d, daily weight gain of weaning piglets was increased linearly ($P=0.047$); while the optimal daily weight gain was achieved when the intake of true digestible Thr was 5.9 g/d (See Table 2). Meanwhile, as the intake was increased, the feed conversion was improved ($P=0.002$). Likewise, the optimal feed conversion was achieved when the intake was 5.9 g/d. There is no effect of true digestible Thr level on the average daily feed intake of piglets ($P=0.822$). Serum urea N level was decreased when the intake of true digestible Thr was increased from 4.1 to 5.0 g/d (linear, $P=0.009$); while serum urea N level was increased when the intake was increased from 5.0 to 6.6 g/d (quadratic, $P=0.015$).

3.2 Effects of the intake of true digestible Thr on serum urea N level of weaning piglets

It has been shown in Table 3 that serum Thr level was significantly increased ($P=0.001$) as the intake of true digestible Thr was increased from 4.1 to 6.6 g/d on both Day 14 and 28; while the level of serum His, Ile and Val were decreased when the intake was increased ($P=0.001$).

3.3 Effects of the intake of true digestible Thr on the concentration of serum anti albumin egg IgG in weaning piglets

On Day 28, the concentration of serum anti albumin egg IgG was increased as the intake of true digestible Thr was increased ($P=0.002$) and the highest concentration was achieved when the intake was 6.6 g/d.

Table 4 Variation of the concentration of serum anti albumin egg IgG[a]

	True digestible Thr level (g/kg)					SEM[1]	*P*-value	
	5.3	5.8	6.5	7.5	8.5		Linear	Quadratic
Day 21	1.9	2.2	2.6	2.3	2.6	0.21	0.094	0.184
Day 28	3.3[c]	3.5[c]	4.4[c]	4.1[c]	6.2[b]	0.39	0.002	0.006

[a] The ratio of serum anti albumin egg IgG level on Day 21 or Day 28 to the level on Day 14.

[b, c] The difference between values in the same row with the same superscript or without superscript is considered as not significant (P>0.05).

[1] SEM: The standard error of the mean.

4 Discussions

The main aim of this experiment is to study the effects of dietary true digestible Thr level on the growth performance, blood indices and immune functions of 10-25 kg piglets. Experimental diets were formulated based on the true ileal digestibility of corn, peanut meal and whey powder measured in previous experiment (Wang et.al., 2007). True digestible Thr level in each treatment diet was 5.3, 5.8, 6.5, 7.5 and 8.5 g/kg respectively and the actual intake of true digestible Thr of pigs was 4.1, 4.4, 5.0, 5.9 and 6.6 g/d respectively.

The maximum daily weight gain and feed conversion were achieved when the intake of true digestible Thr was 5.9 g/d. Similar results have been reported by Lenehan et.al., (2004) that the requirement of true digestible Thr of 10-20 kg piglets was 5.6 g/d when the true digestible Lys level was 11.0 g/d. However, this result is lower than 6.3 g/d which is the estimate of pigs in the same growing phase by NRC (1998). It has been known that the true digestible Thr requirement recommended by NRC (1998) is the value estimated from the predicting model in which the maintenance and the protein accretion are met. However, the factors - difference of the techniques in these two methods or other factors affecting nutrient requirement (genotype, sex, health status, age, diet composition, stock density etc) - resulting in the difference between Thr requirement obtained in this experiment and NRC estimate are not clear.

It has been reported by Coma et.al., (1995) that amino acids requirements can be estimated by the change of serum urea N level induced by the change of dietary amino acid level. Rosell and Zimmerman (1985) have suggested that plasma urea N level was decreased when amino acid requirement reached the estimated value when the maximum N utilization and the minimal urea synthesis were achieved. It indicates that the Thr requirement of pigs is the intake of true digestible Thr when the lowest serum urea N level is achieved. Therefore, it can be deduced that the true digestible Thr requirement in this experiment is 5.0 g/d when estimating using serum urea N level.

Serum free Thr level was significantly increased when the intake of true digestible Thr was increased from 4.1 to 5.9 g/d and then was increased slowly as the intake was increased further.

The similar changing trend of serum free Thr level has been reported by other researches (Mitchell et.al., 1968; Lewis et.al., 1980). Corresponding to the increase of the serum free Thr level, serum free His, Ile and Val level were decreased. The similar decreasing trends of serum free Ile and Val have been reported in studies on Thr requirement by Lewis and Peo (1986). Rosell and Zimmerman (1985) have reported that as a certain amino acid was supplemented, the decrease of certain plasma free amino acids level could indicate their deficiency in diets. Therefore, the supplementation of Thr in Thr-deficient diet can improve the amino acid pattern and increase the protein efficiency of protein synthesis by other amino acids and thus decrease their blood cycle level.

Due to the large difference of serum anti egg albumin IgG between individual pigs, the ratio of the data obtained after the egg albumin injection (Day 21 and 28) to the basal data obtained before the injection (Day 14) was used as the standard to measure the change of serum anti egg albumin IgG level in this experiment. Serum anti egg albumin IgG level was increased as the intake of true digestible Thr was increased. When the highest level was achieved, the true digestible Thr requirement of weaning piglets (6.6 g/d) was higher than the requirement (5.9 g/d) for the maximal growth. Li et.al., (1999) have reported the similar results in growing pigs. They have suggested that Thr requirement for the maximum antibody production and IgG level was higher than the requirement for the maximum growth in 17-31 kg growing pigs.

It has been confirmed that Thr is the main component of plasma γ- globulin in poultry, rabbits and the human beings (Smith & Greene, 1947; Crumpton & Wilkinson, 1963; Tenenhouse & Deutsch, 1966). There is a great amount of Thr in intestinal mucus protein in which Thr accounts for 30% of entire amino acid composition and the deficiency of Thr may result in the decrease of the protein synthesis in intestinal mucus in rats (Faure et.al., 2005). Ball (2001) has suggested that piglets fed Thr-deficient diets were more likely to get diarrhea. All relevant researches have shown that Thr may play an important role in protecting intestinal health and immune functions of intestinal mucosa.

The similar conclusion has been drawn in researches in poultry. Bhargava et.al., (1971) have reported that the Thr requirement of chickens which were affected by New Castle virus was more to meet the optimal antibody production than to meet the growth performance. It has been indicated by Dozier et.al., (2001) that the increase of dietary Thr level may decrease the incidence of sudden death syndrome in cocks. Keshavarz and Austic (1995) have suggested that poultry fed diets with deficient Thr were more sensitive to tumor and plasmodium.

5 Conclusions

5.1 The maximum growth performance can be achieved when the intake of true digestible Thr is 5.9 g/d in 10-25 kg piglets. Thr requirement to meet the optimal immune functions is higher than the requirement to meet the optimal growth performance.

5.2 Thr plays an important role in the adjustment of immune functions of weaning piglets.

Reference

References are available on request.