Original Research Article

Optimal sulfur amino acid to lysine ratio for post weaning piglets reared under clean or unclean sanitary conditions

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ABSTRACT

Two 14-day experiments, each with 90 (Duroc × [Yorkshire × Landrace]; 7.3 ± 0.6 kg) piglets, were conducted to determine the optimum sulfur amino acid (SAA) to lysine (Lys) ratio (SAA:Lys) for piglets when reared under clean or unclean sanitary conditions using performance and non-performance response criteria. Piglets were randomly assigned to the following dietary treatments. The basal diet contained 1.18% standardized ileal digestible (SID) Lys, and the SAA:Lys was 52%. In diets 2 to 5, the basal diet was supplemented with 4 graded levels of DL-Met to make SAA:Lys of 56%, 60%, 64% and 68%. In Exp. 1, piglets were housed in disinfected clean room. In Exp. 2, piglets were housed in a room previously occupied by other pigs and was not disinfected. On the last day, blood was collected to measure plasma urea nitrogen (PUN) and one pig per pen was euthanized to collect jejunal tissue to measure villus height (VH), crypt depth (CD), and VH:CD. In Exp. 1, increasing SAA:Lys linearly and quadratically increased VH and VH:CD ($P < 0.05$). In Exp. 2, increasing SAA:Lys linearly increased ($P < 0.05$) VH and VH:CD and linearly and quadratically decreased PUN ($P < 0.05$). Estimated PUN and VH-based optimum SAA:Lys requirements for clean and unclean sanitary condition were 60%, 63% and 66%, respectively.

1. Introduction

Methionine (Met) requirement varies depending on pig's body weight, sex, and health status. Cysteine (Cys) and methionine form the dietary sulfur amino acids (SAA) that are used for protein synthesis. Methionine is metabolized to Cys, and for 10-kg pigs, 50% of the required dietary SAA can be provided by Cys (Chung and Baker, 1992a). In piglets, 30% of the total dietary Met is used by the splanchnic tissue, as a source of energy, for synthesis of muco-proteins and mucin, antioxidants and for maintenance of redox potential (Stipanuk, 2004; Stoll and Burrin, 2006). Some of the SAA

is utilized by the gut commensal bacteria which prevent pathogenic microbes from attaching to the intestinal wall (Dahiya et al., 2007).

Unsanitary housing conditions cause moderate immune system activation in piglets (Le Floc'h et al., 2006) thus allocating amino acids (AA) towards an immune response rather than protein accretion. Unsanitary conditions could also introduce foreign substances to the gut leading to increased mucin production and intestinal thickness; thereby increase the allocation of SAA towards maintenance requirement. Thus, to avoid growth depression under poor sanitary condition, the pig would either increase feed intake or dietary SAA requirement. Increasing SAA requirement eventually increases the standardized ileal digestible (SID) SAA to lysine (Lys) ratio (SAA:Lys).

The current requirement for SID SAA:Lys for 5-to-12-kg piglets ranges from 50% to 60% (Chung and Baker, 1992b, 1992c; Gaines et al., 2005; Dean et al., 2007; NRC, 2012). These optimum SAA:Lys are based on performance parameters and so far no estimates have been made using non-performance parameters besides plasma urea nitrogen (PUN). The villus height (VH), crypt depth (CD) and the VH to CD ratio (VH:CD) have been used as indicators of gut

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integrity. These indicators of gut integrity are affected by the health status of the pig as well as the dietary nutrient content. Since a good fraction of the dietary SAA is partitioned towards the maintenance of optimum gut health, its content in the diet would directly affect the VH and CD. Thus, the VH, CD and VH:CD were used as non-performance response criteria in determining the optimal dietary SAA:Lys.

It was hypothesized that piglets raised under unclean housing condition will have higher SAA requirement and consequently have higher SID SAA:Lys than piglets raised under clean housing conditions. Thus, the objective of this study was to determine optimum SID SAA:Lys for piglets raised under clean or unclean housing conditions using performance and non-performance response criteria.

2. Materials and methods

2.1. Animal care

The use of pigs and experimental procedures were reviewed and approved by the Animal Care Committee of the University of Manitoba, protocol number F10/041/2. Animals were cared for according to the standard guidelines of the Canadian Council on Animal Care (CCAC, 2009).

2.2. Animals and experimental design

Male and female pigs were obtained from Glenlea Research Station, University of Manitoba. The experiments were conducted for 13 or 14 days at T. K. Cheung Center for Animal Research, University of Manitoba. For each experiment, 90 piglets (Duroc × [Yorkshire × Landrace]; initial average BW = 7.3 ± 0.63 kg) weaned at 21 ± 1 days with 6 replicate pens each containing 3 pigs (1.8 m × 1.2 m) were used. A common starter diet containing 12 g/kg SID Lys and 180 g/kg crude protein was fed for 5 days before the start of experiments. Animals were randomly assigned to 5 dietary treatments including: wheat—corn—soybean meal-based diet containing 11.8 g/kg SID Lys and SID SAA:Lys of 52%. The remaining 4 diets contained the basal diet and SID SAA:Lys of 56%, 60%, 64% and 68% (Table 1). The 11.8 g/kg SID Lys was marginally limiting according to Kahindi et al. (2014a) who reported the SID Lys requirement for 7 to 16 kg piglet fed antibiotic growth promotor (AGP)-free diets was 13.2 g/kg. The graded levels of SID SAA:Lys were attained by replacing corn starch with of DL-Met and the contents of essential AA were similar for all diets and balanced to meet the ideal amino acid ratio for protein accretion (Chung and Baker, 1992c). However, the analyzed Lys contents for diets 3, 4 and 5 were higher (12.4 g/kg) than calculated values. Piglets were allowed free access to feed and water. The pigs were housed in temperature-controlled room with initial temperature of 30 °C that was reduced by 1 °C per week.

Piglets and feeders were weighed on days 6 and 13 for Exp. 1 and weekly in Exp. 2 to determine average daily gain (ADG), average feed intake (ADFI), and gain to feed ratio (G:F). The G:F was calculated on per pen basis by dividing ADG by ADFI. Blood samples were collected from one pig per pen via jugular vein-puncture on days 0 and 13 or 14 into 10 mL heparinized vacutainers tubes (BD Vacutainer, Franklin Lakes, NJ). Blood samples were centrifuged at 1,600 × g for 15 min at 4 °C to harvest plasma for determination of PUN using blood urea colorimetric slides (Vitros, Rochester, NY). The piglets were monitored for incidences of diarrhea, and the severity of diarrhea was assessed using the fecal consistency scoring method of Marquardt et al. (1999). Fecal consistency scoring was (1 = normal; 2 = soft feces; 3 = mild diarrhea and 4 = severe diarrhea). Air quality status in the 2 rooms was analyzed

Table 1
Composition of experimental diets (as-fed basis).

Item	SID SAA to Lys ratio				
	52%	56%	60%	64%	68%
Ingredients, g/kg					
Wheat	602.3	602.3	602.3	602.3	602.3
Corn	100.0	100.0	100.0	100.0	100.0
Soybean meal (46% CP)	208.2	208.2	208.2	208.2	208.2
Vegetable oil	36.6	36.6	36.6	36.6	36.6
Corn starch	5.00	4.50	4.00	3.60	3.10
Monocalcium phosphate	13.9	13.9	13.9	13.9	13.9
Limestone	11.8	11.8	11.8	11.8	11.8
NaCl	3.20	3.20	3.20	3.20	3.20
Mineral—vitamin premix [1]	10.0	10.0	10.0	10.0	10.0
L-lysine-HCl	4.90	4.90	4.90	4.90	4.90
L-threonine	1.80	1.80	1.80	1.80	1.80
L-tryptophan	0.20	0.20	0.20	0.20	0.20
L-valine	2.10	2.10	2.10	2.10	2.10
DL-methionine	0.00	0.50	1.00	1.40	1.90
Calculated nutrient composition, g/kg or as specified					
NE, MJ/kg	10.4	10.4	10.4	10.4	10.4
CP	213.9	213.9	213.9	213.9	213.9
SID lysine	11.8	11.8	11.8	11.8	11.8
SID methionine	2.80	3.30	3.80	4.20	4.70
SID cysteine	3.30	3.30	3.30	3.30	3.30
SID methionine + Cysteine	6.10	6.60	7.10	7.50	8.00
SID threonine	7.70	7.70	7.70	7.70	7.70
SID tryptophan	2.60	2.60	2.60	2.60	2.60
SID isoleucine	7.10	7.10	7.10	7.10	7.10
SID valine	8.30	8.30	8.30	8.30	8.30
SID arginine	11.6	11.6	11.6	11.6	11.6
SID Phenylalanine	9.00	9.00	9.00	9.00	9.00
SID SAA:Lys, %	52.0	56.0	60.0	64.0	68.0
SID methionine:SAA, %	45.9	50.0	53.5	56.0	58.8
Total Ca	8.00	8.00	8.00	8.00	8.00
Available P	4.50	4.50	4.50	4.50	4.50
Analyzed nutrient composition, g/kg					
Total lysine	13.1	13.2	13.7	13.7	13.7
SID lysine	11.8	11.8	12.4	12.4	12.4
Total methionine + Cysteine	6.90	7.50	8.10	8.40	8.80
SID methionine + Cysteine [2]	6.10	6.60	7.20	7.50	7.90

SID = standardized ileal digestible; SAA = sulfur amino acid; Lys = lysine; NE = net energy.

[1] Supplied the following per kilogram of diet: 8,250 IU of vitamin A, 835 IU of vitamin D3, 40 IU of vitamin E, 25 μg of vitamin B12, 4 mg of vitamin K, 25 mg of niacin, 600 mg of choline, 12 mg of riboflavin, 200 μg of biotin, 4.5 mg of pyridoxine, 4 mg of folic acid; 2 mg of thiamin, 50 mg of Mn, 150 mg of Zn, 120 mg of Fe, 25 mg of Cu, 0.35 mg of Se, 0.4 mg of I.

[2] Corrected for the analyzed amino acid contents in the diets, and the calculated ratios from analyzed nutrients were 51%, 56%, 58%, 60% and 64%.

3 times each week. Hydrogen sulphide (H_2S) was measured using a JEROME 631-X machine (Arizona Instrument Corporation, Phoenix, AZ) and ammonia (NH_3) was measured using detector tube (RAE Systems, San Jose, CA). Air samples were collected at pig's height from 3 different places of the room.

One pig with median body weight per pen was slaughtered on days 13 and 14 for Exp. 1 and 2, respectively, to determine jejunal morphology such as VH, CD and VH:CD. The slaughtered piglets were initially anesthetised by an intramuscular injection of ketamine:xylazine (20 mg/kg:2 mg/kg; Bimeda-MTC Animal Health Inc., Cambridge Ontario, Canada) and then killed by an intravenous injection of sodium pentobarbital (50 mg/kg of BW; Bimeda-MTC Animal Health Inc.). The abdominal cavity was exposed by midline laparotomy. A 1-cm section of the mid jejunum was collected, rinsed with ice-cold phosphate buffed saline solution and stored in 10% buffered formalin to fix the villi and the crypts. The sections were processed for histological examination using the standard hematoxylin and eosin method. Villus height (the tip of the villus to the crypt—villus junction) and CD (the crypt—villous junction to the base) were measured

on 10 intact, well-oriented villi per specimen using a compound light microscope equipped with a video camera.

In Exp. 1, piglets were housed in a room maintained as per standard operating procedures of the University of Manitoba's T. K. Cheung Center for Animal Research. In Exp. 2, the piglets were introduced to rooms previously occupied by pigs in Exp. 1 without cleaning the rooms for 1 week after the experimental period to allow manure build up. Additionally, manure slurry from a sow herd (5 kg/pen) was spread on the expanded metal floor before piglets were introduced into the room to further enhance unclean conditions. The spread of manure slurry (5 kg/pen) from the same source was repeated 1 week after the introduction of the piglets because the manure had passed through the expanded metal floor. This room was not cleaned during the experimental period.

2.3. Diet and ingredient analyses

Amino acid concentrations in ingredients and diet samples were determined by ion-exchange chromatography with post-column derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 3 mol/L HCL for 24 h at 110 °C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110 °C (Commission Directive, 2000).

2.4. Data analyses

Data were analyzed using Proc Mixed procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC). The data were analyzed as a completely randomized design and pen was considered the experimental unit for ADG, ADFI, G:F and fecal scores, while piglet was experimental unit for PUN and histomorphological data. The covariates used were initial pen average BW for ADG, day 0 PUN for day 13 or 14 PUN, and final individual BW for histomorphological parameters. Effects of increasing SAA:Lys on performance parameters were analyzed using linear and quadratic polynomial contrasts. The ADG, G:F, VH, CD, VH:CD, and PUN data were fitted to segmented regression (Kaps and Lamberson, 2009) to estimate the optimum SAA:Lys.

Statistical significance level was claimed at $P < 0.05$ and a trend at $0.05 < P < 0.10$.

3. Results

In Exp. 1, increasing dietary SID SAA:Lys quadratically increased ($P < 0.05$) ADG in week 2 (Table 2) but showed not effect on the overall ADG. Increasing SID SAA:Lys ratio linearly increased in week 2 and overall ADFI ($P < 0.05$). Villus height and VH:CD were linearly and quadratically increased ($P < 0.05$) with increasing SID SAA:Lys. However, CD was not significantly affected (Table 4). There was a trend towards a linear increase in PUN with increasing SID SAA:Lys. Ammonia and H_2S concentrations ranged between 25 and 32 mg/kg and 0.06 to 0.07 mg/kg, respectively (Table 5). For all the response criteria apart from ADG, the optimum SAA:Lys was 60% (Table 6). Using ADG the optimum SAA:Lys was 61%.

In Exp. 2, increasing SID SAA:Lys linearly reduced ($P < 0.05$) ADFI but had no effect on the ADG and G:F (Table 3). Increasing SAA:Lys linearly and quadratically decreased ($P < 0.05$) PUN and linearly increased ($P < 0.05$) VH and VH:CD (Table 4). Ammonia concentration was between 32 and 38 mg/kg, whereas H_2S concentration was between 0.14 and 0.20 mg/kg (Table 5). The ADG values were fitted to segmented regression but they failed to converge. Therefore, PUN and VH were used as response criteria. The estimated optimum SAA:Lys were 58%, 63%, and 66%, when using G:F, PUN and VH, as response criteria.

4. Discussion

Post weaning period is a critical time for piglet survival as they are exposed to several stresses. Their gastro intestinal barrier function is not well developed and piglets often experience diarrhea caused by pathogenic infection particularly when reared under commercial production. Environmental stressors under unsanitary condition are expected to cause diarrhea and reduce piglet growth as reported by Kahindi et al. (2014b). However, there were no incidences of diarrhea or observable changes in piglets' health in this study. This could be attributed to piglets' predisposal to maternal feces and that the feces could have had low levels of pathogenic microbes. Addition of manure from sow herd and the unclean environment during the trial did not only make the room filthy but also increased NH_3 and H_2S concentration. Toxic levels of

Table 2
Effect of SID SAA to Lys ratios on body weight, average daily gain, average feed intake, and gain to feed ratio of weaned piglets raised under clean condition (Exp. 1).

| Item | SID SAA to Lys ratio | | | | | SEM | P-value | |
	52%	56%	60%	64%	68%		Linear	Quadratic
BW, kg								
d 0	7.45	7.48	7.47	7.45	7.47	0.25	–	–
d 13	10.6	10.9	11.1	10.9	11.0	0.16	0.209	0.291
Average daily gain, g								
d 1 to 6	139	141	174	148	163	17.2	0.307	0.620
d 7 to 13	342	368	381	403	364	14.2	0.096	0.032
d 1 to 13	248	263	279	278	271	11.9	0.116	0.177
Average daily feed intake, g								
d 1 to 6	258	241	292	256	256	13.3	0.776	0.299
d 7 to 13	479	513	536	566	560	21.9	0.005	0.378
d 1 to 13	368	378	426	405	409	14.7	0.028	0.151
Gain to feed ratio, g/kg								
d 1 to 6	545	590	594	572	640	57.0	0.358	0.922
d 7 to 13	714	717	712	713	649	23.0	0.091	0.152
d 1 to 13	674	700	694	686	665	20.0	0.631	0.227

SID = standardized ileal digestible; SAA = sulfur amino acid; Lys = lysine.

Table 3
Effect of SID SAA:Lys on body weight, average daily gain, average feed intake, and gain to feed ratio of weaned piglets raised under unclean condition (Exp. 2).

Item	SID SAA to Lys ratio					SEM	P-value	
	52%	56%	60%	64%	68%		Linear	Quadratic
BW, kg								
d 0	7.24	7.14	7.13	7.13	7.19	0.10		
d 14	11.1	11.1	11.5	11.4	10.6	0.31	0.583	0.117
Average daily gain, g								
d 1 to 7	194	208	228	236	184	23.3	0.901	0.118
d 8 to 14	359	357	368	374	306	31.6	0.384	0.268
d 1 to 14	277	282	298	305	245	22.7	0.586	0.119
Average daily feed intake, g								
d 1 to 7	346	327	318	356	278	18.1	0.079	0.290
d 8 to 14	555	531	559	531	457	31.8	0.070	0.203
d 1 to 14	450	429	438	444	367	22.2	0.046	0.182
Gain to feed ratio, g/kg								
d 1 to 7	558	633	722	658	659	54.0	0.200	0.151
d 8 to 14	644	672	658	703	666	32.0	0.488	0.566
d 1 to 14	612	660	682	682	662	27.0	0.172	0.142

SID = standardized ileal digestible; SAA = sulfur amino acid; Lys = lysine.

Table 4
Effect of SID SAA to Lys ratios on faecal score, plasma urea nitrogen and jejunal VH, CD and VH:CD of weaned piglets raised under clean and unclean conditions.

Item	SID SAA to Lys ratio					SEM	P-value	
	52%	56%	60%	64%	68%		Linear	Quadratic
Plasma urea nitrogen, mmol/L								
Clean								
d 0	3.51	3.68	3.53	2.74	3.82	0.47	0.820	0.510
d 13	4.34	3.87	4.73	4.53	4.71	0.20	0.089	0.814
Unclean								
d 0	3.66	3.55	3.36	3.48	3.40	0.40	0.648	0.809
d 14	4.61	4.22	3.66	3.36	3.92	0.26	0.014	0.041
Histomorphology								
Clean								
VH, μm	475	538	622	553	571	26.1	0.015	0.021
CD, μm	216	220	213	209	218	4.78	0.536	0.548
VH:CD	2.20	2.42	2.93	2.64	2.63	0.14	0.020	0.031
Unclean								
VH, μm	485	537	531	534	560	22.7	0.027	0.451
CD, μm	222	225	221	210	207	5.95	0.030	0.400
VH:CD	2.19	2.37	2.41	2.71	2.71	0.14	0.006	0.828
Average fecal score[1]								
Clean	1.34	1.33	1.25	1.21	1.14	0.14	0.223	0.896
Unclean	1.34	1.42	1.26	1.40	1.15	0.10	0.192	0.366

SID = standardized ileal digestible; SAA = sulfur amino acid; Lys = lysine; VH = villus height; CD = crypt depth; VH:CD = villus height to crypt depth ratio.
[1] Fecal consistency scoring was: 1 = normal; 2 = soft feces; 3 = mild diarrhea; 4 = severe diarrhea.

Table 5
Air quality status as depicted by concentration of ammonia and hydrogen sulphide (mg/kg) in the clean and unclean rooms.

Item	H₂S				NH₃			
	Clean	SE	Unclean	SE	Clean	SE	Unclean	SE
Day 0	0.06		ND		<5		ND	
Week 1	0.06	0.011	0.14	0.003	32	1.667	32	2.778
Week 2	0.07	0.006	0.20	0.004	25	3.632	38	1.667

ND = not determined.

NH_3 and H_2S would cause growth retardation by reducing feed intake and causing respiratory system discomfort (Ni et al., 2000). However, the H_2S concentration throughout the study period was below the 10 mg/kg level that affects pig growth (Kim et al., 2008). The recommended maximum NH_3 concentration in a swine barn is 20 mg/kg (Donham, 2000). However, pigs can tolerate concentration as higher as 40 mg/kg (Jones et al., 1997). Hence, it is possible that piglets in the unclean sanitary condition could have acclimatized to higher NH_3 concentrations and avoided feed intake depression.

Because there was time difference between the two experiments, the data were analyzed separately rather than as a factorial experiment. Also, no significant differences were observed for the performance data under both conditions. However, the data was still fit to non-linear regression and the r^2 was used in determining reliability of the findings. Baker (1986) argued that rather than linearity or differences between 2 points, data should be fitted to a response curve in order to obtain an optimal value.

Diet formulation using ideal AA ratio for protein accretion is essential because it minimizes feed inefficiencies due to excesses or inadequate dietary AA. The recommended SAA:Lys for protein accretion in healthy 5-to-11-kg pigs are 58% and 55%, respectively (NRC, 2012). However, SAA:Lys of 55% may not be sufficient for optimal performance of growing pigs at different body weight. Gaines et al. (2005) estimated based on ADG a SAA:Lys of 59% for 8-to-19-kg pigs and 60% for 8-to-26-kg pigs. The SAA:Lys of 58% was reported optimum for growth and feed efficiency of 6- to 12-kg pigs (Dean et al., 2007) and 11- to 26-kg pigs (Yi et al., 2006). Earlier work by Chung and Baker (1992c) on 10-kg piglets reported ideal

Table 6
The estimated SAA to Lys ratios from clean and unclean experiments.

Item	Estimates at 95% confidence level		SEM	R^2
	Estimate	Model		
Broken-lines (clean)				
ADG	61.1%	$Y = 46.33 + 3.88 \times ADG - 5.63 \times (ADG - 61.09) \times (ADG - 61.09)$	0.16	99.9
VH	60.0%	$Y = -476 + 18.22 \times VH - 24.45 \times (VH - 60) \times (VH - 60)$	5.93	80.4
VH:CD	60.0%	$Y = -2.33 + 0.3086 \times VH:CD - 0.1186 \times (VH:CD - 60) \times (VH:CD - 60)$	3.22	91.6
PUN	60.0%	$Y = 1.76 + 0.045 \times PUN - 0.021 \times (PUN - 60) \times (PUN - 60)$	6.54	40.3
Broken-lines (unclean)				
VH	65.9%	$Y = 317.3 + 3.53 \times VH + 1.46 \times (VH - 65.9) \times (VH - 65.9)$	3.26	72.4
G:F	58.4%	$Y = -12 + 12 \times G:F - 14.5 \times (G:F - 58.4) \times (G:F - 58.4)$	1.16	97.9
PUN	63.4%	$Y = 10.81 - 0.118 \times PUN + 0.26 \times (PUN - 63.4) \times (PUN - 63.4)$	0.49	99.5

SAA = sulfur amino acid; Lys = lysine; ADG = average daily gain; VH = villus height; CD = crypt depth; VH:CD = villus height to crypt depth ratio; PUN = plasma urea nitrogen; G:F = gain to feed ratio.

SAA:Lys for protein accretion to be 60%, however Chung and Baker (1992b) had reported SAA:Lys of 50% for 5- to 10-kg pig. Based on performance parameters, the optimal SID SAA:Lys was 61% and 58% for clean and unclean conditions, respectively. These values were comparable with the findings of Chung and Baker (1992c), Gaines et al. (2005) and Dean et al. (2007) but higher than that of NRC (2012) for 7- to 11-kg pigs.

Increasing SAA:Lys linearly increased ADFI of piglets raised under clean condition. On the contrary, the ADG was not affected by increasing SID SAA:Lys, hence the G:F was similar for all dietary treatments. Similarly, Chung and Baker (1992b) observed an increase in ADFI and no improvement in feed efficiency with increasing SAA:Lys in 5- to 10-kg pigs. Lack of response in G:F can be attributed to minimal improvement in ADG even with higher dietary SAA:Lys. The optimal SAA:Lys under clean condition was 61%, 60% and 60% for ADG, PUN and VH:CD, respectively. Therefore, under clean condition, the optimal SAA:Lys was similar regardless of the response criteria used. The SID SAA requirement for ADG was 10.89 mg/g gain for piglets under clean condition. This level is higher than 8.7 mg SAA/g gain reported for 5- to 10-kg pigs (Chung and Baker, 1992b) and 10.2 mg SAA/g gain for 6- to 12-kg pigs (Dean et al., 2007) but similar to 10.87 mg SAA/g gain reported by NRC (2012).

Under unclean condition, ADFI was similar for all SID SAA:Lys except for the highest SID SAA:Lys (68%) where depression of ADFI and ADG occurred. Excess Met beyond the optimal SID SAA:Lys in piglet diet can reduce feed intake (Harper et al., 1970; Pearson and Carr, 1979; Iyayi et al., 2014). Depression in feed intake has been attributed to AA imbalances caused by excess dietary Met. Reduction in feed intake as the SAA:Lys increased also resulted in a similar overall G:F. This could be postulated that the SAA were used for the synthesis of acute phase protein but not for growth as indicated by the reduced PUN and improved VH:CD as SAA:Lys increased. The gut utilizes about 30% of the dietary Met (Stoll and Burrin, 2006) for production of energy, cysteine synthesis, production of antioxidants, mucin, enterocytes cell proliferation and maintenance of tissue redox state (Stipanuk, 2004). The VH and VH:CD were increased by 22% from the lowest to the highest SAA:Lys. Similarly, Kaewtapee et al. (2010) and Bauchart-Thevret et al. (2009) reported increased VH as a result of increasing Met content in pigs. Therefore, dietary Met content affects intestinal health and that deficiency in Met reduces enterocytes formation leading to shorter villi. In the present study, the optimum SID SAA:Lys differed depending on the response criteria and were 58%, 63% and 66% for G:F, PUN and VH, respectively. These ratios resulted in SID SAA requirements of 10.44, 11.01 and 11.32 mg/g gain using G:F, PUN and VH, respectively, as response criteria.

Under unclean housing condition, PUN concentration was significantly reduced when SAA:Lys was higher. This result was consistent with that of Owusu-Asiedu et al. (2003) and Kiarie et al. (2009) where elevated PUN concentration was reported in piglets challenged with enterotoxigenic *Escherichia coli* K88$^+$. This is so because unsanitary condition leads to moderate immune system activation in piglets (Le Floc'h et al., 2006) and immune system activation in turn leads to muscle protein breakdown to release amino acid for the synthesis of acute phase protein (Wannemacher, 1977). Muscle protein breakdown also results in an increased PUN concentration. Hence, PUN concentration was low when SAA:Lys ratio was higher because the piglets do not need to breakdown muscle protein when dietary AA requirement is sufficient.

Healthy gut is very important for the overall wellbeing of an animal (Bischoff, 2011), and it is a function of normal gut structure such as VH, CD, and VH:CD. It is commonly believed that an increased VH and a decreased CD is positively correlated to the digestive and absorptive functions in the gastrointestinal tract of animals, accounting for an enlarged absorptive area and a reduced tissue turnover rate (Munyaka et al., 2012; Shang, 2014). To this end, VH was higher in piglets fed a diet contained higher SAA:Lys showing an improved gut health when SAA:Lys was increased.

The higher SAA:Lys for VH in piglets raised under unclean sanitary condition indicate that, SAA requirement for gut tissue development could be higher in piglets when sanitary condition is poor. Therefore, exposure of piglets to foreign substances or pathogens, through feces introduced from elsewhere, could increase the requirement of SAA for development of gut barrier function.

5. Conclusion

Plasma urea nitrogen and VH-based estimated optimal SAA:Lys for post weaning piglets raised under clean or unclean conditions are 60% and 63%, and 66%, respectively. Therefore, in situation where sanitary condition is poor, it is prudent to increase the dietary SAA content above the requirement for growth.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgments

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