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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):lysine (Lys) ratio 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 ratio was 52%. In diets 2 to 5, the basal diet was supplemented with 4 graded levels of DL-Met to make SAA:Lys ratio 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 ratio linearly and quadratically increased VH and VH:CD ($P < 0.05$). In Exp. 2, increasing SAA:Lys ratio 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:lysine (Lys) ratio.

The current requirement for SID SAA:Lys ratio 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 ratios 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:CD ratio 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 ratio were used as non-performance response criteria in determining the optimal dietary SAA:Lys ratio.

It was hypothesized that piglets raised under unclean housing condition will have higher SAA requirement and consequently have higher SID SAA:Lys ratio than piglets raised under clean housing conditions. Thus, the objective of this study was to determine optimum SID SAA:Lys ratio 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 ratio of 52%. The remaining 4 diets contained the basal diet and SID SAA:Lys ratios 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 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 ratio. 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

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

Item	SID SAA: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 D_3, 40 IU of vitamin E, 25 μg of vitamin B_{12}, 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%.

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