EFFICACY OF L-LYSINE SULFATE AS SUPPLEMENT IN SWINE DIETS

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ABSTRACT

A study was conducted to determine the efficacy of supplementing L-lysine sulfate on the growth performance, nitrogen balance and carcass characteristics of swine. Eighteen pigs (initial BW: 11.8±0.51 kg, Landrace x Large White x Duroc) were randomly allotted to two dietary treatments using a completely randomized design. Dietary treatments were corn-soybean meal-based diets supplemented with either L-lysine monohydrochloride (Treatment 1) or L-lysine sulfate (Treatment 2). Experimental diets were formulated to be fed in a 4-phase diet series. The supplemental lysine sources were added to provide 0.195, 0.165, 0.250 and 0.189% of total lysine in the pre-starter, starter, grower, and finisher diets, respectively. Four barrows (~60 days of age) from each treatment were used in a nitrogen balance trial. Six representative animals (~150 days of age) per treatment were slaughtered and evaluated for carcass characteristics. Replacing L-lysine monohydrochloride with L-lysine sulfate in pre-starter to finisher diets did not affect growth performance, nitrogen balance and carcass characteristics. Economic analysis showed that feed cost efficiency may be improved using L-lysine sulfate. Based on the prices of feed ingredients during the study, a PhP 0.81 saving per kilogram gain in weight was attained when L-lysine sulfate was used which could be attributed to its lower price per kilogram. L-Lysine sulfate may be effectively used as an alternative to L-lysine monohydrochloride as a supplemental lysine source in swine diets.

Keywords: L-lysine monohydrochloride, L-lysine sulfate, swine diets

INTRODUCTION

The development of feed grade supplemental amino acids allows nutritionists to formulate diets with the ideal amino acid profile for better performance. Commercially available amino acids have paved the way for easier formulation of diets for optimal growth of animals through efficient utilization of feed nutrients. The reduction in dependence of animal producers to expensive protein concentrates, such as fish meal and soybean meal, has been made possible

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because of these feed supplements.

Lysine, being one of the nine essential amino acids in animal nutrition, is recognized as the first limiting amino acid in swine diets. Most of the cereal grains used in animal feeding, such as corn, are deficient in this essential amino acid. As such, it has been a common practice for decades to supplement amino acids, such as crystalline lysine to provide sufficient amino acids to swine diets. The use of these amino acid supplements may not only be valuable in the aspects of nutrition and economics but also of the environment. Currently, an increasing concern on the negative impact of livestock production has been raised. With the use of amino acid supplements, it could be possible to formulate low-pollution diets and reduce the excessive excretion of nitrogen in the manure to the environment by avoiding nutrient oversupply (Han and Lee, 2000).

The most commonly used lysine supplement in the formulation of animal feeds is L-lysine HCl (L-lysine monohydrochloride), which contains about 78.8% free lysine (Pham *et al*., 2010). A newer supplemental lysine source, L-lysine sulfate, contains about 50% lysine (Liu *et al*., 2007). L-lysine sulfate is a product of bio-fermentation technology but produced by a different post-fermentation process. For its production, the fermentation broth is not separated from the bacterial biomass and not transferred to the hydrochloric acid salt (Ju *et al*., 2008), making the process less complex and producing less waste. It consists of the entire fermentation broth which is conditioned by spray drying and granulation (Eggeling *et al*., 2006). L-lysine sulfate is cheaper and contains other amino acids, as well as sulfur and phosphorus (Ju *et al*., 2008; Wang *et al*., 2007) which are not normally present in L-lysine HCl. L -lysine sulfate was also found to be comparable to L-lysine HCl in terms of relative biological value (Smiricky-Tjardes *et al*., 2004). The objective of this study was to determine the effects of supplementing swine diets with L-lysine sulfate, specifically in terms of growth performance, nitrogen balance and carcass characteristics.

MATERIALS AND METHODS

An experiment was conducted from August 2010 to February 2011 at the University Animal Farm, Animal and Dairy Sciences Cluster, UPLB. A total of 18 triple crossbreed pigs (Landrace x Large White x Duroc) with an average weight of 11.8±0.51 kg were randomly allotted to two treatments using a completely randomized design. Each treatment had nine replicates. The dietary treatments were corn-soybean meal-based diets supplemented with either L-lysine monohydrochloride (Treatment 1) or L-lysine sulfate (Treatment 2). Experimental diets were formulated to be fed in 4 phases from d0 to 16 (pre-starter), d17 to d41 (starter), d42 to d63 (grower) and d64 to d90 (finisher) to correspond with approximate BW ranges of 12-23, 23-43, 43-64 and 64-86 kg (Tables 1 to 4). The pre-starter, starter, grower, and finisher diets were formulated to contain 1.40, 1.20, 1.09 and 0.88% total lysine, respectively. The supplemental lysine sources were added to provide 0.195, 0.165, 0.25 and 0.189% of total lysine in the pre-starter, starter, grower and finisher diets, respectively. Diets were formulated using ingredient values from the PHILSAN Feed Reference Standards (2003). Pigs were allowed *ad libitum* access to water and diets during the feeding trial.

Table 1. Ingredient composition and calculated nutrient analysis of pre-starter diets.

Ingredient, %	Treatment 1 L-lysine HCl	Treatment 2 L-lysine sulfate
Yellow corn	48.59	48.46
Soybean meal	28.39	28.39
Skimmilk powder	5.00	5.00
Rice bran D1	5.00	5.00
Whey powder	5.00	5.00
Limestone	1.87	1.87
Monodicalcium phosphate	1.56	1.56
Coco oil	3.50	3.50
Salt	0.44	0.44
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.10	0.10
Choline chloride	0.04	0.04
L-lysine monohydrochloride	0.25	0.00
L-lysine sulfate	0.00	0.38
DL-methionine	0.10	0.10
L-threonine	0.11	0.11
Copper sulfate	0.02	0.02
Total	100.00	100.00
Calculated Analysis:		
Metabolizable energy, kcal/kg	3250.00	3250.00
Crude protein, %	20.00	20.00
Crude fat, %	6.32	6.32
Calcium, %	1.08	1.08
Available phosphorus, %	0.50	0.50
Total phosphorus, %	0.75	0.75
Total lysine[3], %	1.40	1.40
Total methionine + cystine, %	0.77	0.77

[1]Per kilogram vitamin premix contains: Vitamin A 6,000,000 i.u.; Vitamin D_3 1,000,000 i.u.; Vitamin E 6,000 mg; Vitamin K 1,250 mg; Vitamin B_1 500 mg; Vitamin B_2 2,500 mg; Vitamin B_6 500 mg; Vitamin B_{12} 80 mg; Biotin 25 mg; Calcium pantothenate 3,500 mg; Nicotinic acid 10,000 mg; Excipient q.s. ad 1,000 grams.

[2]Per kilogram mineral premix contains: Potassium iodide 500 mg; Cobalt sulfate 200 mg; Ferrous sulfate 18,000 mg; Copper sulfate 2,600 mg; Zinc oxide 25,500 mg; Calcium carbonate 10,000 mg; Excipient q.s. ad 1,000 grams.

[3]L-lysine monohydrochloride and L-lysine sulfate were added to provide 0.195% of total lysine in experimental diets.

Table 2. Ingredient composition and calculated nutrient analysis of starter diets.

Ingredient, %	Treatment 1 L-lysine HCl	Treatment 2 L-lysine sulfate
Yellow corn	49.18	49.07
Soybean meal	26.66	26.66
Rice bran D1	12.65	12.65
Limestone	1.50	1.50
Monodicalcium phosphate	2.00	2.00
Coco oil	4.00	4.00
Molasses	3.00	3.00
Salt	0.50	0.50
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.10	0.10
Choline chloride	0.04	0.04
L-lysine HCl	0.21	0.00
L-lysine sulfate	0.00	0.32
DL-methionine	0.07	0.07
L-threonine	0.07	0.07
Copper sulfate	0.02	0.02
Total	100.00	100.00
Calculated Analysis:		
Metabolizable energy, kcal/kg	3200.00	3200.00
Crude protein, %	18.30	18.30
Crude fat, %	6.63	6.63
Calcium, %	0.94	0.94
Available phosphorus, %	0.52	0.52
Total phosphorus, %	0.93	0.93
Total lysine[3], %	1.20	1.20
Total methionine + cystine, %	0.66	0.66

[1]Per kilogram vitamin premix contains: Vitamin A 6,000,000 i.u.; Vitamin D_3 1,000,000 i.u.; Vitamin E 6,000 mg; Vitamin K 1,250 mg; Vitamin B_1 500 mg; Vitamin B_2 2,500 mg; Vitamin B_6 500 mg; Vitamin B_{12} 80 mg; Biotin 25 mg; Calcium pantothenate 3,500 mg; Nicotinic acid 10,000 mg; Excipient q.s. ad 1,000 grams.

[2]Per kilogram mineral premix contains: Potassium iodide 500 mg; Cobalt sulfate 200 mg; Ferrous sulfate 18,000 mg; Copper sulfate 2,600 mg; Zinc oxide 25,500 mg; Calcium carbonate 10,000 mg; Excipient q.s. ad 1,000 grams.

[3]L-lysine monohydrochloride and L-lysine sulfate were added to provide 0.195% of total lysine in experimental diets.

Table 3. Ingredient composition and calculated nutrient analysis of grower diets.

Ingredient, %	Treatment 1 L-lysine HCl	Treatment 2 L-lysine sulfate
Yellow corn	45.17	45.00
Soybean meal	18.76	18.76
Rice bran D1	26.94	26.94
Limestone	1.04	1.04
Monodicalcium phosphate	1.89	1.89
Coco oil	2.00	2.00
Molasses	3.00	3.00
Salt	0.50	0.50
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.10	0.10
Choline chloride	0.04	0.04
L-lysine HCl	0.32	0.00
L-lysine sulfate	0.00	0.49
DL-methionine	0.11	0.11
L-threonine	0.11	0.11
Copper sulfate	0.02	0.02
Total	100.00	100.00
Calculated Analysis:		
Metabolizable energy, kcal/kg	3100.00	100.00
Crude protein, %	16.30	16.30
Crude fat, %	5.29	5.29
Calcium, %	0.75	0.75
Available phosphorus, %	0.50	0.50
Total phosphorus, %	1.07	1.07
Total lysine[3], %	1.09	1.09
Total methionine + cystine, %	0.60	0.60

[1]Per kilogram vitamin premix contains: Vitamin A 6,000,000 i.u.; Vitamin D_3 1,000,000 i.u.; Vitamin E 6,000 mg; Vitamin K 1,250 mg; Vitamin B_1 500 mg; Vitamin B_2 2,500 mg; Vitamin B_6 500 mg; Vitamin B_{12} 80 mg; Biotin 25 mg; Calcium pantothenate 3,500 mg; Nicotinic acid 10,000 mg; Excipient q.s. ad 1,000 grams.

[2]Per kilogram mineral premix contains: Potassium iodide 500 mg; Cobalt sulfate 200 mg; Ferrous sulfate 18,000 mg; Copper sulfate 2,600 mg; Zinc oxide 25,500 mg; Calcium carbonate 10,000 mg; Excipient q.s. ad 1,000 grams.

[3]L-lysine monohydrochloride and L-lysine sulfate were added to provide 0.195% of total lysine in experimental diets.

Table 4. Ingredient composition and calculated nutrient analysis of finisher diets.

Ingredient, %	Treatment 1 L-lysine HCl	Treatment 2 L-lysine sulfate
Yellow corn	42.04	41.91
Soybean meal	13.34	13.34
Rice bran D1	33.28	33.28
Limestone	1.22	1.22
Monodicalcium phosphate	1.43	1.43
Coco oil	2.31	2.31
Molasses	5.00	5.00
Salt	0.50	0.50
Vitamin premix[1]	0.10	0.10
Mineral premix[2]	0.10	0.10
Choline chloride	0.30	0.30
L-lysine HCl	0.24	0.00
L-lysine sulfate	0.00	0.37
DL-methionine	0.06	0.06
L-threonine	0.06	0.06
Copper sulfate	0.02	0.02
Total	100.00	100.00
Calculated Analysis:		
Metabolizable energy, kcal/kg	3100.00	3100.00
Crude protein, %	14.40	14.40
Crude fat, %	5.82	5.82
Calcium, %	0.75	0.75
Available phosphorus, %	0.40	0.40
Total phosphorus, %	1.04	1.04
Total lysine[3], %	0.88	0.88
Total methionine + cystine, %	0.48	0.48

[1]Per kilogram vitamin premix contains: Vitamin A 6,000,000 i.u.; Vitamin D_3 1,000,000 i.u.; Vitamin E 6,000 mg; Vitamin K 1,250 mg; Vitamin B_1 500 mg; Vitamin B_2 2,500 mg; Vitamin B_6 500 mg; Vitamin B_{12} 80 mg; Biotin 25 mg; Calcium pantothenate 3,500 mg; Nicotinic acid 10,000 mg; Excipient q.s. ad 1,000 grams.

[2]Per kilogram mineral premix contains: Potassium iodide 500 mg; Cobalt sulfate 200 mg; Ferrous sulfate 18,000 mg; Copper sulfate 2,600 mg; Zinc oxide 25,500 mg; Calcium carbonate 10,000 mg; Excipient q.s. ad 1,000 grams.

[3]L-lysine monohydrochloride and L-lysine sulfate were added to provide 0.195% of total lysine in experimental diets.

Growth performance

The pigs were weighed individually at the start of the experiment and at the end of each feeding phase. Feed disappearance was also determined by weighing the orts. Average daily gain, average daily feed intake, feed efficiency (F:G) and number of days to reach 85 kg were calculated.

Nitrogen balance

Four barrows weighing 22.5±2.3 kg (~60 days of age) on the average were used for the nitrogen balance trial. Each pig, housed in individual metabolism crates, was fed a weighed amount of the treatment diet twice a day. Each experimental period consisted of 7 days with a 3-day adaptation period to the experimental diets and a 4-day collection period. The total amount of feeds consumed, feces and urine produced were recorded daily during the collection period. Urine was collected in a container with 400 ml of 10% HCl (v/v) to prevent loss of ammonia and microbial growth as specified in the methods of Lee *et al*. (1998). Feces were collected using a pan attached to the crate and then dried in an oven for 72 hours. Daily collections were pooled together from which representative samples (approximately 20% of daily collections) were obtained. Collected feces and urine were placed in sealed containers and stored in a freezer (-18℃). Nitrogen absorption, nitrogen retention, net protein utilization and biological value of feed protein were calculated.

Carcass characteristics

After the feeding trial, six representative animals per treatment were randomly selected and slaughtered at a mean live weight of 87.5±2.3 kg (~140 days of age) after an average fasting of 12 hours. Carcasses were subjected to standard carcass evaluation to determine backfat thickness, loin eye area, fat-free lean weight and percentage of fat-free lean.

Feed cost efficiency

The feed cost per kilogram weight gain was calculated based on the price of raw materials during the time of the experiment.

Chemical analysis

The two lysine supplements were subjected to amino acid analysis to determine their amino acid content. The amino acid content was determined following acid hydrolysis with 6N HCl for 48 hours, using an amino acid analyzer (Shimadzu LC-10 AVP HPLC System). The nutrient composition of feeds was determined by proximate and mineral analysis following AOAC procedures (1990). Feeds, collected feces and urine were subjected to Kjeldahl nitrogen determination as specified in AOAC (1990).

Statistical analysis

Data on growth performance, nitrogen balance and carcass characteristics were collected and subjected to a t-test using SAS (SAS Institute, 1989). Each pig was considered as an experimental unit. In all analyses done, a probability of $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The analyzed lysine content of L-lysine HCl was 78.1% whereas L-lysine sulfate contained 51.2% lysine (Table 5). However, amino acids such as methionine, cysteine, threonine, tryptophan, arginine and isoleucine were present in L-lysine sulfate but not in L-lysine HCl, which is consistent with the findings of Ju *et al*. (2008) and Wang *et al*. (2007). There are differences in the post-fermentation processing of the two lysine supplements (Rodehutscord *et al*., 2000). The production of L-lysine sulfate includes the fermentation biomass that provides the other amino acids, whereas none of the fermentation products are included in L-lysine HCl (Jackson, 2001). The other amino acids present in L-lysine sulfate are co-products since their biosynthesis coincide with some of the preliminary steps in the synthesis of lysine (Dale and Park, 2004). Therefore, the use of L-lysine sulfate in swine diets may be advantageous because of the additional amino acids it provides, which are mostly indispensable.

Table 5. Amino acid content of L-lysine monohydrochloride and L-lysine sulfate.

Amino acid, %	L-lysine HCl	L-lysine sulfate
Lysine	78.1	51.2
Methionine	nd	0.33
Cysteine	nd	0.09
Threonine	nd	0.25
Tryptophan	nd	0.12
Arginine	nd	0.35
Isoleucine	nd	0.31

nd – not detected

No significant differences were observed in all the performance parameters measured in pigs fed diets supplemented with L-lysine HCl compared with L-lysine sulfate (Table 6). These results were in agreement with Smiricky-Tjardes *et al*. (2004) and Liu *et al*. (2007).

There were no differences (P>0.05) in N intake, amount of fecal and urinary N excreted, N absorbed and N retained between pigs fed the dietary treatments (Table 7). Consequently, the net protein utilization and biological value of protein did not differ between dietary treatments. These results are in agreement with studies comparing the two lysine sources in pigs (Ju *et al*., 2008; Liu *et al*., 2007), broilers (Wang *et al*., 2007) and rainbow trout (Rodehutscord *et al*., 2000). A nitrogen balance trial is more sensitive in assessing the adequacy of amino acid sources than growth trials in pigs (Figueroa *et al*., 2002). Taking the results of nitrogen balance and growth performance together, it is quite evident that both lysine sources have similar lysine availability and, thus, L-lysine sulfate could be used as an alternative to L-lysine monohydrochloride.

Table 6. Growth performance of pigs fed diets supplemented with either L-lysine monohydrochloride or L-lysine sulfate.

Parameter	Treatment 1 L-lysine HCl Supplementation	Treatment 2 L-lysine sulfate Supplementation	SEM
Number of replicates	9	9	
Initial body weight, kg ^{ns}	11.75	11.78	0.261
Prestarter			
Final body weight, kg ^{ns}	22.610	23.110	0.725
Average daily gain, kg ^{ns}	0.639	0.667	0.044
Average daily feed intake, kg ^{ns}	1.000	1.036	0.067
F:G^{ns}	1.573	1.562	0.080
Starter			
Final body weight, kg ^{ns}	42.490	44.210	1.885
Average daily gain, kg ^{ns}	0.795	0.844	0.057
Average daily feed intake, kg ^{ns}	1.712	1.703	0.124
F:G^{ns}	2.170	2.017	0.078
Grower			
Final body weight, kg ^{ns}	63.600	64.370	2.592
Average daily gain, kg ^{ns}	0.960	0.916	0.043
Average daily feed intake, kg ^{ns}	2.559	2.458	0.148
F:G^{ns}	2.673	2.673	0.095
Finisher			
Average daily gain, kg ^{ns}	0.955	0.906	0.064
Average daily feed intake, kg ^{ns}	2.866	2.857	0.172
F:G ^{ns}	3.029	3.175	0.178
Overall			
Days to reach 85 kg ^{ns}	87	88	3.728
Average daily gain, kg ^{ns}	0.850	0.841	0.036
Average daily feed intake, kg ^{ns}	2.103	2.060	0.087
F:G^{ns}	2.481	2.453	0.084

^{ns} Not significant (P>0.05)

The source of supplemental lysine did not affect the carcass characteristics of pigs (Table 8). This indicates that replacing L-lysine monohydrochloride with L-lysine sulfate in swine diets did not negatively affect carcass leanness. Nutritional factors, such as adequacy of amino acid and energy intake, affect protein and fat deposition in pigs (Pettigrew and Esnaola, 2001). For pigs to efficiently utilize dietary amino acids for lean deposition, it is necessary that amino acids are available, balanced and adequate relative to their requirements. Consequently, amino acid-deficient diets may increase available energy for fat deposition which directly affects carcass leanness.

Based on the prices of feed ingredients during the conduct of study, the feed cost efficiency of diets supplemented with L-lysine sulfate were PhP 0.49, PhP 3.28,

Table 7. Nitrogen balance of pigs with supplementation of either L-lysine monohydrochloride or L-lysine sulfate.

Parameter	Treatment 1 L-lysine HCl Supplementation	Treatment 2 L-lysine sulfate Supplementation
Nitrogen intake, g/day^{ns}	43.20	44.93
Nitrogen excreted in urine, g/day^{ns}	17.01	22.53
Nitrogen excreted in feces, g/day^{ns}	3.36	3.99
Nitrogen absorbed, g/day ^{ns}	26.19	22.41
Nitrogen retained, g/day ^{ns}	22.82	18.41
Net protein utilization ^{ns}	0.53	0.41
Biological value of feed protein, % ^{ns}	87.00	81.00

^{ns} Not significant (P>0.05).

Table 8. Carcass characteristics of pigs fed diets supplemented with either L-lysine monohydrochloride or L-lysine sulfate.

Parameter	Treatment 1 L-lysine HCl Supplementation	Treatment 2 L-lysine sulfate Supplementation	SEM
Backfat thickness, cm ^{ns}	2.41	2.44	0.117
Loin eye area, cm$^{2\ ns}$	31.87	30.77	0.239
Fat-free lean weight, kg ^{ns}	33.13	33.71	1.036
% Fat-free lean ^{ns}	55.03	54.91	0.731

^{ns} Not significant (P>0.05).

and PhP 0.45 less than pre-starter, starter, and grower diets supplemented with L-lysine monohydrochloride, respectively (Table 9). However, in the finisher diets, feed cost efficiency was greater in L-lysine monohydrochloride than L-lysine sulfate-supplemented diets. Overall, the use of L-lysine sulfate resulted in a savings of about PhP 0.81 per kilogram body weight gain compared to using L-lysine monohydrochloride. This means that use of L-lysine sulfate is a more cost efficient approach in meeting the lysine requirement of swine, provided that the price of L-lysine sulfate is competitive.

CONCLUSION

Based on the results of the study, it can be concluded that L-lysine sulfate may be effectively used as an alternative to L-lysine monohydrochloride as a supplemental lysine source in swine diets. Since similar responses were seen in terms of growth performance, nitrogen balance and carcass characteristics, it would be cost-practical to use L-lysine sulfate as a supplemental source of lysine in swine feeding provided that its price is competitive.

Table 9. Economic analysis of supplementing diets with either L-lysine monohydrochloride or L-lysine sulfate.

Parameter	Treatment 1 L-lysine HCl Supplementation	Treatment 2 L-lysine sulfate Supplementation
Prestarter		
Feed cost, PhP/kg	23.81	23.66
Feed cost efficiency, PhP/kg BWG	37.46	36.97
Starter		
Feed cost, PhP/kg	19.92	19.80
Feed cost efficiency, PhP/kg BWG	43.21	39.93
Grower		
Feed cost, PhP/kg	18.30	18.12
Feed cost efficiency, PhP/kg BWG	48.91	48.46
Finisher		
Feed cost, PhP/kg	18.34	18.20
Feed cost efficiency, PhP/kg BWG	55.55	57.78
Overall		
Total feed consumed, kg	184.79	180.33
Total feed cost, PhP	3547.54	3441.76
Total body weight gain, kg	74.49	73.53
Feed cost efficiency, PhP/kg BWG	47.63	46.82

Price of feed ingredients (in PhP): Yellow corn 14.50; Soybean meal US Hi-pro 26.00; Skimmilk powder 45.00; Rice bran D1 12.80; Whey powder 56.00; Limestone 2.00; Monodicalcium phosphate 26.50; Coco oil 54.00; Molasses 11.80; Iodized salt 5.00; Vitamin premix 1300.00; Mineral premix 130.00; Choline chloride 77.00; L-lysine HCl 130.00; L-lysine sulfate 52.00; DL-methionine 265.00; L-threonine 150.00; Copper sulfate 125.00.

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