Kansas Agricultural Experiment Station Research Reports

Volume 10
Issue 6 *Swine Day*

Article 31

2024

Effect of Benzoic Acid, Myristic Acid, and Aspergillus Niger on the AME and N Retention in Grow-Finishing Pigs

Diego A. Lopez
Kansas State University, diegolopez@k-state.edu

Mason J. Engnell
Kansas State University, mengnel2@k-state.edu

Allison K. Blomme
Kansas State University, ablomme@k-state.edu

See next page for additional authors

Recommended Citation

Lopez, Diego A.; Engnell, Mason J.; Blomme, Allison K.; Minson, Carter; Friesen, W. Garrett; Wilson, Victoria C.; Stark, Charles R.; and Paulk, Chad B. (2024) "Effect of Benzoic Acid, Myristic Acid, and Aspergillus Niger on the AME and N Retention in Grow-Finishing Pigs," *Kansas Agricultural Experiment Station Research Reports*: Vol. 10: Iss. 6. https://doi.org/10.4148/2378-5977.8646

Effect of Benzoic Acid, Myristic Acid, and Aspergillus Niger on the AME and N Retention in Grow-Finishing Pigs

Authors

Diego A. Lopez, Mason J. Engnell, Allison K. Blomme, Carter Minson, W. Garrett Friesen, Victoria C. Wilson, Charles R. Stark, and Chad B. Paulk

K-STATE Research and Extension

SWINE DAY 2024

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2024

Effect of Benzoic Acid, Myristic Acid, and *Aspergillus Niger* on the AME and N Retention in Grow-Finishing Pigs

Diego A. Lopez,[1] Mason J. Engnell,[1] Allison K. Blomme,[1] Carter Minson,[1] W. Garrett Friesen,[1] Victoria C. Wilson,[1] Charles R. Stark,[1] and Chad B. Paulk[1]

Summary

An experiment was conducted to determine the effect of benzoic acid, myristic acid, and *Aspergillus niger* on the digestibility of DM and GE, and the concentration of DE and ME in diets fed to growing pigs. A total of 10 barrows (DNA 200 × 400, DNA; initially 75.2 ± 2.27 lb.) were allotted to a replicated 5 × 5 Latin square design with five treatments and five periods for a total of 10 replicate pigs per treatment. Pigs were individually housed in metabolic crates equipped with a feeder, drinker, and a wire mesh floor. Pigs were fed dietary treatments for a 5-day adaptation period followed by a 5-day collection period. During collection, a screen and a urine pan were installed below the floor of the crate to allow for the total and separate collection of feces and urine. A basal corn-soybean meal, wheat middlings and DDGS-based diet was formulated as a negative control and a corn soybean meal-based diet was formulated as positive control. Three additional diets were formulated by adding one source of benzoic acid, myristic acid, or *Aspergillus niger* to the negative control. Therefore, a total of five diets were formulated. The positive control diet had the greatest ($P < 0.05$) ATTD of GE and DM, ME and DE compared to the negative control diet with or without feed additives. There was no evidence of difference between ATTD of DM and GE between the negative control diet and the negative control diets with either benzoic acid, myristic acid, or *Aspergillus niger*. However, the negative control diet containing myristic acid had increased ($P < 0.05$) DE concentration compared to the negative control. There was a tendency for the negative control diets containing benzoic acid and *Aspergillus niger* to have increased ($P < 0.15$) concentration of DE compared to the negative control diet without feed additives. Similarly, there was a tendency of increased ($P < 0.15$) concentration of ME in diets containing added myristic acid and benzoic acid compared to the negative control diet. There was no evidence of difference ($P > 0.15$) in ME concentration between the negative control diet with and without *Aspergillus niger*. The positive control diet had the greatest ($P < 0.05$) N retention and N digestibility as percentage of intake compared to all negative control treatments. However, there was no evidence of differences between the negative control diet and the negative control treatments containing the feed additives. This data suggests that the inclusion of myristic acid and benzoic acid has the potential to increase the concentration of ME in diets with high fiber concentrations without impacting N retention.

[1] Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA

Introduction

Feed additives are commonly used in the swine industry to accomplish several goals such as improving the sanitary status of the herd, promoting growth performance, and increasing the release of nutrients. Some feed additives, such as organic acids, acidifiers, and dietary cultures of fungus (e.g. *Aspergillus niger*) are traditionally used in diets of weaning pigs as alternatives of antibiotics. Myristic acid is an organic acid that has been included in diets to improve intestinal health and growth performance.[2] Likewise, benzoic acid has been used as an acidifier due to its antimicrobial activity. Lastly, cultures of *Aspergillus niger* are believed to be beneficial in reducing the incidences of intestinal disorders including diarrhea or necrotic enteritis.

These feed additives aim to improve gut health and prevent the incidence of diarrhea. However, another possible effect of the inclusion of these feed additives might be an increase in the digestibility of nutrients and energy in diets fed to pigs.[3] Previous research has been conducted to determine the effect of organic acids, acidifiers, and dietary cultures of fungus on weaning pig's growth performance. However, there is limited research determining the influence of these feed additives on the digestibility of nutrients and energy in growing pigs. Therefore, the objective of this study is to determine the effect of myristic acid, benzoic acid, and *Aspergillus niger* on the digestibility of DM, GE, and N, and the concentrations of DE and ME in diets with a high concentration of fiber fed to growing-finishing pigs.

Materials and Methods

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted in the metabolism room at the Kansas State University Swine Teaching and Research Center, where pigs were individually housed in metabolic crates that allow for the total and separate collection of feces and urine. All crates were equipped with a feeder, a drinker, and a wire mesh floor. During collection, a screen and a urine pan were installed below the floor of the crate.

Animals and diets

A total of 10 barrows (DNA 200 × 400, DNA; initially 75.2 ± 2.27 lb) were used in this experiment. At the start of the experiment, all pigs were weighed and moved into metabolic crates. Each pig was allotted to a replicated 5 × 5 Latin square design with five dietary treatments and five periods of 10 d in each square. Therefore, there were 10 replicate pigs per treatment. During each period, there was a 5-d adaptation period followed by a 5-d collection period. Throughout the experiment pigs had ad libitum access to water and were fed 3.0 times the estimated requirement for maintenance energy (i.e., 197 kcal of ME per kg of BW0.60) divided into two equal meals fed at 0800 and 1700 h.

[2] Choi, H., G. C. Rocha, S. W. Kim. 2024. Impacts of dietary myristic acids on mucosa-associated microbiota in relation to intestinal health and growth parameters of nursery pigs. J. Anim. Sci. 102: 40 -41. doi: 10.1093/jas/skae102.047

[3] Rao, Z-X., M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, J. T. Gebhardt. 2023. Effects of various feed additives on finishing pig growth performance and carcass characteristics: A review. Animals. doi: 10.3390/ani13020200

A basal corn, soybean meal, wheat middlings. and DDGS-based diet was formulated as a negative control and a corn-soybean meal-based diet was also formulated as a positive control. Both diets were formulated to meet the recommended nutrient requirements of grow-finishing pigs (110-165 lb).[4] Three additional diets were formulated by adding one source of benzoic acid, myristic acid, or *Aspergillus niger* to the negative control basal diet. Thus, five dietary treatments were used in this experiment and manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. During manufacturing, samples of the diets and ingredients were collected.

Fecal samples were collected using the marker-to-marker approach.[5] In summary, a screen and a urine pan were installed below the floor of the metabolic crates, and an indigestible marker (ferric oxide) was included in the meal fed in the morning of d 6 to start the collection and in the morning of d 10 to stop the collection. During the collection period, orts were collected daily prior to feeding the morning meal, and urines were collected in buckets that contained 50mL of 6N HCl to preserve the samples. The buckets of urine were weighed daily, and the value was recorded prior to taking a subsample of 20% of the total volume of urine, right after the remaining urine was emptied and HCl was reapplied to the buckets. Fecal, orts, and urine samples were stored at -20°C after collection.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a subsample was taken for chemical analyses. Fecal samples were dried in a forced-air oven at 55°C,[6] finely ground, mixed, and subsampled. Fecal, urine, ingredient, and feed samples were analyzed for dry matter (DM; Method 930,15; AOAC, 2007), N (method 990.03; AOAC, 2007), and GE using bomb calorimetry.

Concentrations of DE and ME in each diet were calculated using the following equations:

$$DE_{Diet}, kcal/lb = GE_{Diet} - GE_{Feces}\,;\, ME_{Diet} = DE_{Diet} - GE_{Urine}$$

where GE_{Diet}, GE_{Feces}, and GE_{Urine} represent the concentration of energy (kcal/lb) in the feed, fecal samples, and urine samples respectively.

The apparent total tract digestibility (ATTD) of each diet was calculated using the following equation:

$$ATTD_{Nutrient}, \% = [(Nutrient_{intake} - Nutrient_{feces}) / Nutrient_{intake}] \times 100$$

where $Nutrient_{intake}$ and $Nutrient_{feces}$ represent the daily intake and fecal output (g/d) of the nutrient of interest, respectively.

[4] NRC, 2012. Nutrient requirements of swine: 11th revised edition. Washington, DC: National Academies Press.

[5] Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. CRC Press, Washington, D.C. p. 903-916

[6] Jacobs, B. M., J. F. Patience, W. A. Dozier, III, K. J. Stalder, B. J. Kerr. 2011. Effects of drying methods on nitrogen and energy concentrations in pig feces and urine, and poultry excreta. J. Anim. Sci. 89 (2624-2630). doi: 10.2527/jas.2010-3768

Lastly, N retention (Nr) was calculated using the following equation:

$$Nr, \% = [(N_{intake} - \{N_{feces} + N_{urine}\}) / N_{intake}] \times 100$$

where N_{intake}, N_{feces}, and N_{urine} represent the daily intake, fecal output, and urine output (g/d) of N, respectively.

Statistical analysis

Experimental data was analyzed using the GLIMIX procedure of SAS (SAS Institute Inc., Cary, NC) using pig as the experimental unit and pig and period as the random effect. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Studentized residuals were calculated for each observation. Observations with a studentized residual outside of ± 3 were considered outliers and removed. Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.15$.

Results and Discussion

Concentrations of nutrients in corn and corn DDGS (Table 1) are in agreement with published data.[7] However, both soybean meal and wheat middlings used in this experiment appear to have a greater concentration of gross energy compared to what was expected. Likewise, the concentration of ADF in wheat middlings was also greater than reported values. In contrast, the concentration of crude protein in soybean meal was lower than reported values. Diet composition is presented in table 2. Analyzed concentrations of nutrients in diets (Table 3) are in agreement with calculated concentrations during the formulation of the diets.

Pigs remained healthy during the experiment, and very little feed refusals were observed. There was an overall treatment effect ($P < 0.001$) for ATTD of DM and GE. Pigs fed the positive control diet had the greatest ($P < 0.05$) ATTD of GE and DM compared to the negative control diets with or without feed additives. The difference can be explained by the increased concentration of fiber in the negative control diet provided by DDGS and wheat middlings. When adding benzoic acid, myristic acid, or *Aspergillus niger* to the negative control, there was no evidence of difference ($P > 0.15$) in ATTD of GE or DM compared to the negative control.

There was an overall treatment effect ($P < 0.001$) for the concentration of DE (as-is or DM basis) and ME (as-is or DM basis). The concentration of DE (as-is or DM basis) was greatest ($P < 0.05$) in the positive control diet compared to the negative control diets with or without feed additives. The negative control diet containing myristic acid had a greater ($P < 0.05$) concentration of DE compared to the negative control diet, indicating that the inclusion of myristic acid could increase the concentration of DE in diets containing DDGS and wheat midds. In addition, there was a tendency ($P < 0.15$) for the benzoic acid, and *Aspergillus niger* diets to have a greater DE concentration than the negative control diet. The concentration of ME (as-is or DM basis) was the greatest ($P < 0.05$) in the positive control diet when compared to the negative control diet with and without the feed additives. Diets containing myristic acid and benzoic acid tended

[7] NRC, 2012. Nutrient requirements of swine: 11th revised edition. Washington, DC: National Academies Press.

($P < 0.15$) to have a greater concentration of ME compared to the negative control diet with diets containing *Aspergillus niger* being intermediate. Therefore, there was no evidence of difference ($P > 0.15$) in concentration of ME between the negative control diet and the negative control diet containing *Aspergillus niger*.

There was an overall treatment effect ($P < 0.001$) for N intake, Fecal N, Urinary N, N retained, N retention as a percent of intake, and N digestibility. The N intake of the pigs fed the positive control diet was the lowest ($P < 0.05$), whereas the pigs fed the diet containing *Aspergillus niger* had the greatest ($P < 0.05$) intake of N, with all other treatments being intermediate. There was no evidence of difference ($P > 0.15$) in the intake of N among the pigs fed the negative control diet and the negative control diets containing myristic acid and benzoic acid. Because diets were not formulated to be isoproteic, these results were expected, as the positive control diets had 2.4% N and the negative control diets ranged from 3.2-3.4%. For fecal N, pigs fed the positive control diet had a decreased ($P < 0.05$) fecal N compared to the pigs fed the other dietary treatments. There was no evidence of difference ($P > 0.15$) in fecal N between the negative control with or without feed additives. Pigs fed the positive control diet had decreased ($P < 0.05$) urinary N when compared to the pigs fed the negative control and negative control with benzoic acid and *Aspergillus niger*. There was no evidence of difference in urinary nitrogen between pigs fed the negative control diet and the negative control diets containing either myristic acid, benzoic acid, or *Aspergillus niger*. For g of N retained per day, the pigs fed the positive control diet had a decreased ($P < 0.05$) retained N (g/d) compared to the pigs fed the negative control with myristic acid, benzoic acid and *Aspergillus niger*. However, there was no evidence of difference between the pigs fed the positive control diet and the negative control diet. This is the result of a lower intake of N in pigs fed the positive control diet. In contrast, the retention of N as a percentage of intake was the greatest ($P < 0.05$) for the positive control diet. No differences were observed between the negative control diet and the negative control with myristic acid, benzoic acid, or *Aspergillus niger*. Lastly, for N digestibility, the positive control diet had the greatest ($P < 0.05$) digestibility of N compared to the negative control diets with or without feed additives. There was no evidence of difference between the negative control diet and the negative control with either myristic acid, benzoic acid, or *Aspergillus niger*. However, the diet containing myristic acid had a decreased N digestibility compared to the dietary treatment containing *Aspergillus niger*.

In conclusion, formulating diets to contain DDGS and wheat midds resulted in a higher concentration of GE, CP, crude fat, ash, crude fiber, NDF, and ADF, as expected. These differences in nutritional composition led to corn and soybean meal-based diets having increased DE and ME concentrations, N retention as a percent of intake, and N digestibility compared to diets containing DDGS and wheat midds. This data also suggests that the inclusion of myristic acid and benzoic acid to corn, soybean meal, DDGS and wheat midds-based diets has the potential to increase the concentration of ME (29 and 28 kcal/lb as-is basis, respective) in diets without impacting the N retention or digestion; however, it does not increase the ME to equal the value of the corn and soybean meal- based (positive control) diet. There was no evidence of improvement in N digestibility or N retention when adding myristic acid, benzoic acid, or *Aspergillus niger* to a corn, soybean meal, DDGS, and wheat midds-based diet.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Analyzed composition of feed ingredients (as-is basis)

Item	Corn	Soybean meal	Corn DDGS	Wheat Middlings
Gross energy, kcal/lb	1,934	2,141	2,213	2,017
Dry matter, %	87.39	88.75	88.05	88.21
Crude protein, %	7.98	44.11	28.16	15.83
AEE, %[1]	3.75	3.90	7.46	2.96
Ash, %	1.17	6.56	5.10	5.45
Crude fiber, %	1.62	4.58	7.92	7.98
NDF, %[2]	8.62	9.94	25.36	36.96
ADF, %[3]	2.67	5.81	12.27	11.00

[1]AEE = Acid-hydrolysis ether extract.
[2]NDF = Neutral detergent fiber.
[3]ADF = Acid detergent fiber.

Table 2. Ingredient composition of experimental diets containing Gutmyria, Benzocal-50, and Probioist (as-fed basis)[1]

Item, %	Negative Control	Positive Control	Myristic acid	Benzoic acid	Aspergillus niger
Corn	36.74	78.24	36.67	36.64	36.69
Soybean meal, 46.5% CP	11.99	18.98	11.99	11.99	11.99
Corn DDGS	30.00	-	30.00	30.00	30.00
Wheat middlings	19.00	-	19.00	19.00	19.00
Calcium carbonate	1.00	0.77	1.00	1.00	1.00
Monocalcium P, 21% P	-	0.55	-	-	-
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Mineral premix[2]	0.15	0.15	0.15	0.15	0.15
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25
L-Lys-HCl	0.40	0.40	0.40	0.40	0.40
DL-Met	-	0.05	-	-	-
L-Thr	0.02	0.10	0.02	0.02	0.02
L-Trp	0.02	0.03	0.02	0.02	0.02
L-Val	-	0.05	-	-	-
Phytase[4]	0.03	0.03	0.03	0.03	0.03
Myristic acid	-	-	0.07	-	-
Benzoic acid	-	-	-	0.10	-
Aspergillus niger	-	-	-	-	0.05
Total	100.00	100.00	100.00	100.00	100.00

[1]Myristic acid, Benzoic acid, and *Aspergillus niger* provided by Insighter Biotech, Guangzhou, China.
[2]Provided the following quantities of minerals per lb of diet: Copper 11,000 ppm, Iodine 198 ppm, Iron 73,413 ppm, Manganese 22,046 ppm, Selenium 198 ppm, Zinc 73,413 ppm.
[3]Provided the following quantities of vitamins per lb of diet: Vitamin A 750,000 IU, Vitamin D 300,000 IU, Vitamin E 8,000 mg, Vitamin B12 6 mg, menadione 600 mg, riboflavin 1,500 mg, d-Pantothenic Acid 5,000 mg, niacin 9,000 mg.
[4]RONOZYE HiPhos 2700, DSM, Parsippany, NJ.

Table 3. Analyzed composition of experimental diets (as-fed basis)

Item	Negative Control	Positive Control	Myristic acid	Benzoic acid	Aspergillus niger
Gross energy, kcal/lb	2,018	1,899	2,049	2,047	2,041
Dry matter, %	87.80	87.68	87.59	87.85	87.40
Crude protein, %	19.80	15.210	19.52	20.32	21.39
Crude fat, %	2.89	1.84	2.56	2.50	5.26
Ash, %	5.07	3.67	5.29	5.23	5.39
Crude fiber, %	5.11	2.19	5.04	5.26	5.31
NDF, %[1]	16.70	7.49	16.80	17.80	16.86
ADF, %[2]	7.09	3.43	7.10	7.18	7.09

[1]NDF = Neutral detergent fiber.
[2]ADF = Acid detergent fiber.

Table 4. Digestible energy (DE), metabolizable energy (ME), and apparent total tract digestibility (ATTD) of gross energy (GE) in negative and positive control diets, and diets containing myristic acid, benzoic acid, and *Aspergillus niger*[1]

Item	Negative Control	Positive Control	Myristic acid	Benzoic acid	Aspergillus niger	SEM	*P*-value
ATTD of DM, %	80.79^{b,x}	91.59^{a,w}	80.60^{b,x}	80.76^{b,x}	80.73^{b,x}	0.411	0.0001
ATTD of GE, %	80.01^{b,x}	90.25^{a,w}	80.53^{b,x}	80.41^{b,x}	80.41^{b,x}	0.500	0.0001
GE intake, kcal/d	8,434^{b,y}	8,032^{c,z}	8,738^{ab,wx}	8,588^{ab,xy}	8,768^{a,w}	781.5	0.0001
GE in feces, kcal/d	1,689^{a,w}	786^{b,x}	1,684^{a,w}	1,680^{a,w}	1,717^{a,w}	138.9	0.0001
GE in urine, kcal/d	369.0	349.6	413.7	398.9	450.9	67.79	0.2314
DE, kcal/lb	1,614^{c,y}	1,714^{a,w}	1,650^{b,x}	1,646^{bc,x}	1,641^{bc,x}	10.144	0.0001
DE, kcal/lb DM[2]	1,838^{c,y}	1,955^{a,w}	1,883^{b,x}	1,873^{bc,x}	1,878^{bc,x}	11.571	0.0001
ME, kcal/lb	1,524^{b,y}	1,636^{a,w}	1,553^{b,x}	1,552^{b,x}	1,537^{b,xy}	14.391	0.0001
ME, kcal/lb DM	1,736^{b,y}	1,865^{a,w}	1,774^{b,x}	1,767^{b,x}	1,759^{b,xy}	16.428	0.0001

$^{a-c}$Means within a row lacking a common superscript letter are different ($P < 0.05$).
$^{w-z}$Means within a row lacking a common superscript letter tend to be different ($P < 0.15$).
[1]A total of 10 barrows (DNA 200 × 400, DNA; initial body weight 75.2 ± 2.27 lb) were used in this experiment. Each pig was allotted to a 5 x 5 replicated Latin square design with five diets and five periods for a total of 10 pigs per treatment.
[2]DM= Dry matter.

Table 5. Nitrogen balance of pigs fed negative and positive control diets, and diets containing myristic acid, benzoic acid, and *Aspergillus niger*

Item	Negative Control	Positive Control	Myristic acid	Benzoic acid	Aspergillus niger	SEM	*P*-value
N intake, g/d[1]	60.59^{b}	46.33^{c}	60.41^{b}	61.89^{b}	66.68^{a}	5.376	0.001
Fecal N, g/d[2]	9.33^{a}	4.67^{b}	9.44^{a}	9.88^{a}	9.64^{a}	0.651	0.001
Urinary N, g/d[3]	13.95^{a}	8.24^{b}	12.89^{ab}	13.55^{a}	15.46^{a}	2.139	0.023
N retained, g/d[4]	37.30^{ab}	33.43^{b}	38.08^{a}	38.46^{a}	41.57^{a}	4.066	0.026
N retention, % of intake[5]	61.09^{b}	71.85^{a}	62.26^{b}	62.22^{b}	62.72^{b}	2.934	0.026
N digestibility, %[6]	84.57^{bc}	89.85^{a}	84.17^{c}	84.45^{bc}	85.41^{b}	0.587	0.001

$^{a-c}$Means within a row lacking a common superscript letter are different ($P < 0.05$).
[1]N intake, g/d = feed intake during collection period, g/d × (feed N content, % ÷ 100)
[2]Fecal N, g/d = feces weight, g/d, air-dry basis × (feces N content, % ÷ 100)
[3]Urinary N, g/d = urine weight, g/d × (urine N content, % ÷ 100)
[4]N retained, g/d = N intake, g/d – Fecal N, g/d – Urinary N, g/d
[5]N retention, % of intake = (N retained g/d ÷ N intake, g/d) × 100
[6]N digestibility, % = Apparent total tract digestibility of N; ((N intake, g/d – Fecal N, g/d) ÷ N intake, g/d) × 100