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## Effect of Enzymatically Fermented Soybean Meal and Lactobacillus Plantarum on Nursery Pig Performance

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# Effect of Enzymatically Fermented Soybean Meal and *Lactobacillus Plantarum* on Nursery Pig Performance

## Abstract

A total 360 pigs (PIC C-29 × 359, initially 12.2 lb) were used in a 45-d trial to determine the effects of enzymatically fermented soybean meal (EFS) and *Lactobacillus plantarum* (LP1) on nursery pig performance. Pigs were allotted by BW and sex, and randomly assigned to 1 of 4 dietary treatments, with 9 replications per treatment. Dietary treatments were arranged in a 2 × 2 factorial with main effects of added EFS (0 vs. 8% replacing soybean meal) and LP1 (0 vs. 0.1%). Experimental diets were fed in two phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 24) with a common diet fed to all pigs from d 24 to 45 post-weaning. From d 0 to 14, pigs fed diets containing EFS had decreased ( $P < 0.05$ ) ADG, ADFI, and d 14 BW compared with pigs fed diets without EFS. However, there were no differences in growth performance observed for LP1. From d 14 to 24, pigs fed diets containing EFS had improved ( $P = 0.035$ ) F/G; however, there were no differences in ADG or ADFI among treatments. Furthermore, no differences in growth performance were observed for LP1. From d 0 to 24, pigs fed the diet containing EFS had a tendency for decreased ( $P = 0.09$ ) ADFI compared to pigs fed diets without EFS; however, no differences were observed for ADG and F/G. In addition, pigs fed diets containing LP1 had a tendency for improved ( $P = 0.06$ ) F/G compared to pigs fed diets without LP1, but no differences were observed for ADG or ADFI. During the common period (d 24 to 45), there was a tendency for increased ( $P = 0.08$ ) ADFI for pigs previously fed diets containing LP1 compared to pigs previously fed diets without LP1; however, there were no differences detected for ADG or F/G. Overall (d 0 to 45), a LP1 × EFS interaction was detected for F/G ( $P < 0.01$ ) where LP1 and EFS individually each improved ( $P < 0.05$ ) F/G, but when combined, F/G was similar to the control diet. No differences were observed for the main effects of EFS or LP1. In conclusion, pigs fed EFS had decreased ADFI which led to lower growth rates immediately post-weaning. Interestingly, the addition of LP1 and EFS in nursery diets improved F/G when fed independently from one another, but when combined, no growth benefit was reported.

## Keywords

enzymatically fermented soybean meal, *Lactobacillus plantarum*, nursery pig

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## Cover Page Footnote

Appreciation is expressed to Dr. Jason Swell and Terry Waugh, Nutraferma Inc., Sioux City, IA, and Brent Ratliff, Kindstrom-Schmoll Inc., Eden Prairie, MN, for their technical support and to Nutraferma Inc., Sioux City, IA, for their partial financial support. Appreciation is expressed to Julie Salyer, Dr. Brad James, and Lorene Parkhurst, Kalmbach Feeds, for their technical support and expertise in conducting the experiment.

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## Effect of Enzymatically Fermented Soybean Meal and *Lactobacillus Plantarum* on Nursery Pig Performance<sup>1,2</sup>

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### Summary

A total 360 pigs (PIC C-29 × 359, initially 12.2 lb) were used in a 45-d trial to determine the effects of enzymatically fermented soybean meal (EFS) and *Lactobacillus plantarum* (LP1) on nursery pig performance. Pigs were allotted by BW and sex, and randomly assigned to 1 of 4 dietary treatments, with 9 replications per treatment. Dietary treatments were arranged in a 2 × 2 factorial with main effects of added EFS (0 vs. 8% replacing soybean meal) and LP1 (0 vs. 0.1%). Experimental diets were fed in two phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 24) with a common diet fed to all pigs from d 24 to 45 post-weaning. From d 0 to 14, pigs fed diets containing EFS had decreased ( $P < 0.05$ ) ADG, ADFI, and d 14 BW compared with pigs fed diets without EFS. However, there were no differences in growth performance observed for LP1. From d 14 to 24, pigs fed diets containing EFS had improved ( $P = 0.035$ ) F/G; however, there were no differences in ADG or ADFI among treatments. Furthermore, no differences in growth performance were observed for LP1. From d 0 to 24, pigs fed the diet containing EFS had a tendency for decreased ( $P = 0.09$ ) ADFI compared to pigs fed diets without EFS; however, no differences were observed for ADG and F/G. In addition, pigs fed diets containing LP1 had a tendency for improved ( $P = 0.06$ ) F/G compared to pigs fed diets without LP1, but no differences were observed for ADG or ADFI. During the common period (d 24 to 45), there was a tendency for increased ( $P = 0.08$ ) ADFI for pigs previously fed diets containing LP1 compared to pigs previously fed diets without LP1; however, there were no differences detected for ADG or F/G. Overall (d 0 to 45), a LP1 × EFS interaction was detected for F/G ( $P < 0.01$ ) where LP1 and EFS individually each improved ( $P < 0.05$ ) F/G, but when combined, F/G was similar to the control diet. No differences were observed for the main effects of EFS or LP1. In conclusion, pigs fed EFS had decreased ADFI which led to lower growth rates immediately post-weaning. Interestingly, the addition of LP1 and EFS in nursery

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<sup>2</sup> Appreciation is expressed to Julie Salyer, Dr. Brad James, and Lorene Parkhurst, Kalmbach Feeds, for their technical support and expertise in conducting the experiment.

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diets improved F/G when fed independently from one another, but when combined, no growth benefit was reported.

Key words: enzymatically fermented soybean meal, *Lactobacillus plantarum*, nursery pig

## Introduction

Voluntary feed intake is often low and variable directly after weaning. As a result, research has focused on how nutritional stressors can be overcome to stimulate feed intake and subsequently increase performance (Pluske et al., 1997<sup>4</sup>). Thus, highly palatable and nutrient dense protein sources are commonly added to nursery diets to encourage feed intake. Traditionally, this has been accomplished with the addition of milk and animal-based by-products. However, concern of cost, availability, and bio-security concerns has led many producers to seek alternatives.

One product that has gained significant interest over the years is the use of enzymatically fermented soybean meal (EFS). Enzymatically fermented soybean meal is a product obtained from the fermentation of conventional soybean meal using a mixed culture of bacteria and fungus (Wang et al., 2014<sup>5</sup>). This process can effectively reduce the number of anti-nutritional factors associated with allergenic responses in weaned pigs as well as modify the amino acid profile via microbial synthesis (Hong et al., 2004<sup>6</sup>). Likewise, the use of probiotics has been a major focus within the swine industry in recent years. Probiotics can be defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001<sup>7</sup>). Therefore, it's been proposed that probiotics have the potential ability to influence the microbiota balance and the integrity of the intestinal epithelia (Metzler et al., 2005<sup>8</sup>). Thus, the objective of this study was to evaluate the growth performance of nursery pigs fed EFS and a commercially produced probiotic (*Lactobacillus plantarum*: LP1) independently and together in a commercial research facility.

## Procedures

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Cooperative Research Farm's Swine Research Nursery (Sycamore, OH), which is owned and managed by Kalmbach Feeds, Inc. Each pen had slatted metal floors and was equipped with a 4-hole stainless steel feeder and one nipple-cup waterer for ad libitum access to feed and water. Pens were 5 × 6 ft to allow 3 ft<sup>2</sup> per pig. Nursery rooms were not power washed or disinfected after the previous group of pigs.

<sup>4</sup> Pluske, J.R., D.J. Hampson, and I.H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* 51:215-236.

<sup>5</sup> Wang, Y., X.T. Liu, H.L. Wang, D.F. Li, X.S. Piao, and W.Q. Lu. 2014. Optimization of processing conditions for solid-state fermented soybean meal and its effects on growth performance and nutrient digestibility of weanling pigs. *Livest. Sci.* 170:91-99.

<sup>6</sup> Hong, K. J., C. H. Lee, and S. W. Kim. 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Food* 7:430-434.

<sup>7</sup> FAO/WHO (Food and Agriculture Organization/World Health Organization) 2006. Probiotics in food: Health and nutritional properties and guidelines for evaluation. <ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf>.

<sup>8</sup> Metzler, B., E. Bauer, R. Mosenthin. 2005. Microflora management in the gastrointestinal tract of piglets. *Asian-Aust. J. Anim. Sci.* 18:1353-1362.

A total of 360 pigs (PIC C-29 × 359, initially 12.2 lb) with 10 pigs per pen and 9 replications per treatment were used in a 45-d growth performance trial evaluating the effects of enzymatically fermented soybean meal and *Lactobacillus plantarum* supplementation on the growth performance of nursery pigs. Pigs were weaned at approximately 18 to 20 d and allotted to pens based on initial weight in a completely randomized design to 1 of 4 dietary treatments (Tables 1 and 2). Dietary treatments were arranged in a 2 × 2 factorial with main effects of added EFS (0 vs. 8% replacing soybean meal) and LP1 (0 vs. 0.1%). Pigs and feeders were weighed on d 0, 7, 14, 24, 35, and 45 of the trial to determine ADG, ADFI, and F/G.

Experimental diets were fed in two phases, with the first phase being provided at 5 lb per pig from d 0 to 14. The second phase was fed until pigs reached approximately 25 lb BW (d 24 post-weaning). A common nursery Phase 3 diet was then fed to all pigs for three weeks following the experimental diets (d 24 to 45 post-weaning). All diets were fed in pellet form during the trial.

Samples of treatment protein sources were collected at the feed mill during diet manufacturing. Complete diet samples were obtained from each dietary treatment each wk during the study and composited. Samples were then stored at -4°F until analysis. Composite samples of protein sources and diets were analyzed for DM, CP, ADF, NDF, crude fiber, Ca, P, Cl, Na, ether extract, and starch (Ward Laboratory, Kearney, NE).

Data were analyzed using the PROC GLIMIX procedure in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Dietary treatments were the fixed effect in the analysis. The main effects of LP1 and EFS, as well as their interactions, were tested. Differences between treatments were determined by using least square means, with results considered significant at a  $P$ -value  $\leq 0.05$  and considered a trend  $0.05 < P \leq 0.10$ .

## Results and Discussion

Chemical analysis of experimental diets and the EFS fed during this trial were reasonably consistent with formulated values.

There were no EFS × LP1 interactions observed for the entire study with the exception of overall (d 0 to 45) F/G. From d 0 to 14, pigs fed diets containing EFS had decreased ( $P < 0.05$ ) ADG, ADFI, and d 14 BW compared to pigs fed diets without EFS. Added LP1 had no effect on d 0 to 14 performance. From d 14 to 24, pigs fed diets containing EFS had improved ( $P = 0.035$ ) F/G; however, there were no differences in ADG or ADFI among treatments. Furthermore, no differences in growth performance were observed for LP1.

From d 0 to 24, pigs fed the diet containing EFS had a tendency for decreased ( $P = 0.09$ ) ADFI compared to pigs fed diets without EFS; however, no differences were observed for ADG and F/G. In addition, pigs fed diets containing LP1 had a tendency for improved ( $P = 0.06$ ) F/G compared to pigs fed diets without LP1, but no differences were observed for ADG and ADFI.

During the common period (d 24 to 45), there was a tendency for increased ( $P = 0.08$ ) ADFI for pigs previously fed diets containing LP1 compared to the negative control and negative control with EFS; however, there were no differences detected for ADG or F/G.

Overall (d 0 to 45), an LP1  $\times$  EFS interaction was detected for F/G ( $P < 0.01$ ) where LP1 and EFS each improved ( $P < 0.05$ ) F/G, but when combined, F/G was similar to the control diet. No differences were observed for the main effects of LP1 or EFS.

In conclusion, pigs fed EFS had poorer ADFI which led to poorer growth rates immediately post-weaning. Interestingly, the addition of LP1 and EFS in nursery diets improved F/G when fed independently from one another, but when combined, no growth benefit was reported. A possible explanation for the lack of response could be attributed to the fact that the EFS contained fewer anti-nutritional factors, thus potentially reducing gut inflammation and the opportunity for bacterial overgrowth that LP1 has been recognized to act upon. Nevertheless, the post-weaning period remains a challenge for newly weaned pigs that will continue to warrant research to evaluate specialty ingredients that can maximize feed intake while improving feed efficiency.

**Table 1. Chemical analysis of Phase 1 and 2 diets<sup>1,2</sup>**

Item, %	Control	Control +		
		LP1	EFS	LP1 + EFS
Phase 1 diets				
DM	89.87	90.34	91.86	91.52
CP	24.20	24.60	23.20	24.40
ADF	4.80	4.70	4.70	5.00
NDF	8.00	8.70	8.10	7.90
Crude fiber	2.80	3.40	2.60	2.70
Ca	0.93	0.93	1.02	0.88
P	0.72	0.75	0.65	0.65
Cl	0.58	0.66	0.66	0.64
Na	0.23	0.25	0.23	0.24
Ether extract	4.20	4.00	4.10	3.90
Ash	6.44	6.76	7.09	6.95
Phase 2 diets				
DM	90.07	90.39	89.70	90.73
CP	23.40	24.20	24.80	24.50
ADF	4.00	5.20	5.00	4.70
NDF	7.40	7.10	12.50	7.00
Crude fiber	2.80	2.80	3.90	2.80
Ca	0.87	0.86	0.81	0.83
P	0.57	0.66	0.67	0.58
Cl	0.62	0.64	0.61	0.51
Na	0.23	0.24	0.21	0.23
Ether extract	4.50	4.70	4.60	4.60
Ash	6.34	6.25	5.75	6.21

<sup>1</sup> Complete diet samples were obtained from each dietary treatment each week during the study and composited. Samples of the diets were then submitted to Ward Laboratories, Inc. (Kearny, NE) for analysis.

<sup>2</sup> *Lactobacillus plantarum* and enzymatically fermented soybean meal (Nutraferma, Sioux City, IA).



**Table 2. Chemical analysis of the Phase 3 diet<sup>1</sup>**

Item, %	Common diet
DM	88.40
CP	22.90
ADF	6.50
NDF	13.40
Crude fiber	3.40
Ca	0.92
P	0.70
Cl	0.26
Na	0.19
Ether extract	4.90
Ash	5.24

<sup>1</sup>A composite sample was submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis.

**Table 3. Laboratory analysis of enzymatically fermented soybean meal<sup>1</sup>**

Item, %	EFS <sup>2</sup>
DM	94.56
CP	51.00
ADF	10.10
NDF	12.70
Crude fiber	3.80
Ca	0.60
P	0.77
Cl	0.63
Na	0.03
Ether extract	1.50
Ash	6.94

<sup>1</sup>Proximate analysis for enzymatically fermented soybean meal was analyzed by Ward Labs, Kearney, NE.

<sup>2</sup>Enzymatically fermented soybean meal (Nutraferma, Sioux City, IA).



**Table 4. Ingredient composition of experimental diets<sup>1</sup>**

Ingredient, %	Phase 1		Phase 2		Phase 3
	Control	EFS	Control	EFS	Common
Corn <sup>2</sup>	28.00	28.59	38.09	38.61	52.02
Soybean meal, 46.5% CP	35.03	26.50	36.00	27.50	32.50
Corn DDGS <sup>3</sup>	10.00	10.00	10.00	10.00	10.00
Spray dried whey	21.75	21.75	10.85	10.85	---
EFS <sup>4</sup>	---	8.00	---	8.00	---
Tallow	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.05	1.10	1.15
Monocalcium P, 21% P	0.85	0.75	0.75	0.65	1.10
Salt	0.25	0.25	0.30	0.30	0.40
L-Lys HCL	0.24	0.28	0.23	0.27	0.37
DL-Met	0.15	0.15	0.12	0.12	0.14
L-Thr	0.09	0.09	0.09	0.09	0.15
L-Trp	---	---	---	---	0.01
Phytase <sup>5</sup>	0.01	0.01	0.01	0.01	0.01
Zinc oxide	0.40	0.40	0.26	0.26	---
Choline chloride, 70% liq.	0.04	0.04	0.04	0.04	---
Selenium, 0.6%	0.02	0.02	0.02	0.02	0.02
Trace mineral premix	0.09	0.09	0.09	0.09	0.09
Vitamin premix	0.10	0.10	0.10	0.10	0.05
TOTAL	100	100	100	100	100

*continued*

**Table 4. Ingredient composition of experimental diets<sup>1</sup>**

	Phase 1		Phase 2		Phase 3
	Control	EFS	Control	EFS	Common
Calculated analysis					
Standardized ileal digestible (SID) amino acids, %					
Lys	1.40	1.40	1.35	1.35	1.30
Met:Lys	34	35	33	33	35
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	65	65	65	65	65
Trp:Lys	20	20	20	20	18
Val:Lys	71	71	73	73	69
ME, kcal/lb	1,520	1,524	1,519	1,519	1,515
CP, %	24.55	24.80	24.57	24.83	22.91
Ca, %	0.96	0.96	0.90	0.90	0.92
P, %	0.86	0.84	0.80	0.80	0.81
Available P, %	0.59	0.59	0.50	0.50	0.50

<sup>1</sup>Phase 1 diets were fed for 14 d or to approximately 15 lb BW (5 lb/pig). Phase 2 diets were fed from approximately 15 lb to approximately 25 lb BW. Phase 3 diets were fed d 24 to 45 post-weaning.

<sup>2</sup>*Lactobacillus plantarum* (Nutraferma, Sioux City, IA) was included in the diet at 0.10% at the expense of corn.

<sup>3</sup>Dried distillers grains with solubles.

<sup>4</sup>Enzymatically fermented soybean meal (Nutraferma, Sioux City, IA).

<sup>5</sup>Quantum Blue (AB-Vista Americas, Plantation, FL) provided 227 phytase units (FTU)/lb of diet, with a release of 0.13% available P.

**Table 5. Effect of enzymatically fermented soybean meal and *Lactobacillus plantarum* on nursery pig performance**

	Control <sup>2</sup>	Negative Control +			SEM	Probability, <i>P</i> <
		LP1 <sup>3</sup>	EFS <sup>3</sup>	LP1 + EFS		LP1 × EFS
BW, lb						
d 0	12.2	12.2	12.2	12.2	0.03	0.262
d 14	16.8	16.9	16.5	16.5	0.17	0.814
d 24	24.0	24.3	23.9	24.2	0.30	0.892
d 45	50.2	51.6	50.9	51.3	0.61	0.460
d 0 to 14						
ADG, lb	0.33	0.34	0.31	0.30	0.012	0.617
ADFI, lb	0.37	0.35	0.34	0.34	0.009	0.269
F/G	1.14	1.04	1.11	1.12	0.032	0.124
d 14 to 24						
ADG, lb	0.72	0.75	0.74	0.77	0.023	0.943
ADFI, lb	1.08	1.08	1.05	1.08	0.022	0.595
F/G	1.52	1.46	1.43	1.41	0.032	0.534
d 0 to 24						
ADG, lb	0.49	0.51	0.49	0.50	0.013	0.696
ADFI, lb	0.67	0.66	0.64	0.64	0.012	0.588
F/G	1.36	1.29	1.31	1.30	0.021	0.174
d 24 to 45						
ADG, lb	1.25	1.30	1.28	1.29	0.020	0.375
ADFI, lb	1.79	1.84	1.79	1.85	0.030	0.928
F/G	1.43	1.42	1.40	1.43	0.014	0.113
d 0 to 45						
ADG, lb	0.85	0.88	0.86	0.87	0.014	0.366
ADFI, lb	1.19	1.21	1.18	1.21	0.019	0.837
F/G	1.41 <sup>a</sup>	1.38 <sup>b</sup>	1.37 <sup>b</sup>	1.39 <sup>ab</sup>	0.010	0.007

<sup>ab</sup> Means within the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup> A total of 360 pigs (PIC C-29 × 359) were used with 10 pigs/pen and 9 replications/trt.

<sup>2</sup> Negative control – high soybean meal; first 24 d; Phases 1 and 2.

<sup>3</sup> *Lactobacillus plantarum* (fed from d 0 to 24) and EFS (fed from d 0 to 24) (Nutraferma, Sioux City, IA).

**Table 6. Main Effects of enzymatically fermented soybean meal and *Lactobacillus plantarum* on nursery pig performance<sup>1</sup>**

	LP1 <sup>2</sup>		EFS <sup>2</sup>		SEM	Probability, <i>P</i> <	
	-	+	-	+		LP1	EFS
BW, lb							
d 0	12.2	12.2	12.2	12.2	0.02	0.918	0.424
d 14	16.6	16.7	16.8	16.5	0.12	0.728	0.046
d 24	24.0	24.3	24.1	24.1	0.21	0.246	0.757
d 45	50.5	51.5	50.9	51.1	0.43	0.131	0.785
d 0 to 14							
ADG, lb	0.32	0.32	0.34	0.31	0.009	0.891	0.026
ADFI, lb	0.36	0.35	0.36	0.34	0.006	0.179	0.013
F/G	1.13	1.08	1.08	1.12	0.023	0.159	0.420
d 14 to 24							
ADG, lb	0.73	0.76	0.73	0.76	0.016	0.197	0.269
ADFI, lb	1.07	1.08	1.08	1.07	0.015	0.595	0.434
F/G	1.48	1.43	1.49	1.42	0.023	0.172	0.035
d 0 to 24							
ADG, lb	0.49	0.50	0.50	0.49	0.009	0.365	0.634
ADFI, lb	0.65	0.65	0.66	0.64	0.009	0.786	0.092
F/G	1.33	1.30	1.33	1.30	0.015	0.058	0.246
d 24 to 45							
ADG, lb	1.27	1.30	1.28	1.29	0.014	0.174	0.608
ADFI, lb	1.79	1.85	1.82	1.82	0.022	0.075	0.871
F/G	1.41	1.43	1.42	1.41	0.010	0.291	0.504
d 0 to 45							
ADG, lb	0.85	0.87	0.86	0.86	0.010	0.150	0.969
ADFI, lb	1.18	1.21	1.20	1.19	0.013	0.174	0.703
F/G	1.39	1.39	1.39	1.38	0.007	0.820	0.147

<sup>1</sup> A total of 360 pigs (PIC C-29 × 359) were used for the study.

<sup>2</sup> Negative control – high soybean meal; first 24 d; Phases 1 and 2.

<sup>3</sup> *Lactobacillus plantarum* (fed from d 0 to 24) and enzymatically fermented soybean meal (fed from d 0 to 24) (Nutraferma, Sioux City, IA).