EFFECT OF A WATER-BASED PHYTOGENIC SUPPLEMENT ON GROWTH PERFORMANCE OF NURSERY PIGS FED LOW PROTEIN DIETS

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Jessie Barnes

Oklahoma State University

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Thesis Approved:

Dr. Adel Pezeshki

Thesis Adviser

Dr. Ranjith Ramanathan

Name: JESSIE BARNES

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Abstract:

The objective of the current study was to assess the effects of a phytogenic supplement at three different doses on growth performance of weaned piglets fed with normal or low protein diets. A total of 48 weanling crossbred pigs (Duroc sire line and Large White X Landrace dam) barrows were used. All pigs were weaned at 3-weeks of age and housed in individual pens. In 2 × 3 factorial design, all pigs were randomly assigned into 6 groups (9.1 ± 0.91 kg; 8 pigs/group) receiving a combination of diets with 2 levels of protein (16% and 24% crude protein [CP]) and 3 levels of Herbanimal® supplement (0, 4, and 8 ml/L) for 4 weeks. The groups were included: 1) 24% CP + 0 ml/L Herbanimal® supplement (CON-NS), 2) 24% CP + 4 ml/L Herbanimal® supplement (CON-LS), 3) 24% CP + 8 ml/L Herbanimal® supplement (CON-HS), 4) 16% CP (low protein) + 0 ml/L Herbanimal® supplement (LP-NS), 5) 16% CP + 4 ml/L Herbanimal® supplement (LP-LS), 6) 16% CP + 8 ml/L Herbanimal® supplement (LP-HS). The feed and water intake were measured daily, and the body weight was recorded weekly. Overall, CON-HS had the highest final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), average daily water intake (ADWI), and gain to feed (G:F) ratio. LP-HS had the lowest final BW and ADG compared to other groups. LP-HS and LP-LS had the lowest G:F ratio and were equivalent to each other. Numerically, LP-NS had the lowest ADFI and ADWI. The greatest difference in final BW was detected between CON-HS and LP-HS ($P < 0.05$). Pigs fed CON-HS had the largest body weight gain (BWG) during weeks 1 and 4 while CON-LS had the highest BWG during week 2. Pigs fed with CON-NS and LP-LS had equally higher values in week 3 compared to other groups. The lowest BWG for week 1 was for LP-LS and that for weeks 2-4 was for LP-HS. CON-HS had the largest G:F ratios for weeks 1 and 4 while LP-LS had the largest G:F in week 2. Pigs fed with LP-NS had the highest G:F in week 3 among all groups. LP-NS and LP-LS shared the lowest value for the G:F ratio during week 1 as did CON-LS and LP-LS for week 4. LP-HS had the lowest G:F ratio during weeks 2-3 among all groups. These results indicate that the Herbanimal® supplement improved the growth performance of nursery pigs fed with diets with normal protein levels, but had no positive effect on the growth performance of nursery pigs fed low protein diets.

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INTRODUCTION

Weaning is a highly stressful practice for piglets. This is due to variety of factors including emotional and physiological effects. Nursery pigs tend to suffer from diarrhea and depressed growth performance during weaning.[1]These negative effects decrease feed intake, which then further negatively affect gut health and function.[2] Overall, weaning tends to cause a higher risk for disease, low feed intake, and nutritional disorders.[3] Therefore, researchers seek new approaches to decrease the stress of weaning on the gastrointestinal (GI) tract of piglets without using antibiotics.

The costs associated with weaning stress is $1.95 per 20 lb piglet, $1.75 per 22.5lb piglet, and $1.58 per 25lb piglet.[4] These costs are associated with medications used, veterinarian costs and etc. As a result, for a litter of 13 piglets that weigh an average of 22.5 lbs the average cost due to weaning stress is $22.75. There is a significant increase in mortality and susceptibility to gram-negative bacteria in weaning nursery pigs.[3] The increased death rate increases the costs even more because if a piglet dies from a disease it is being treated for, then not only did the producer incur a cost but also lost any potential gains from that pig.

Weaning has various effects on the overall health of piglets.[s] The immune system of nursery pigs is compromised as they highly rely on sow's milk to acquire immunity as well as for their growth and survival.[5] The transition from liquid diet to a solid food diet with a different composition can have some negative impacts, which impairs the morphology and functions of nursery pig's GI tract.[2] Additionally, reduced feed intake may promote inflammation of the GI tract affecting villus and crypt structure, which then can affect the body's ability to absorb the available nutrients.[2] The immature digestive system in weaned piglets can result in reduced activity of digestive enzymes, impaired intestinal morphology and decreased digestion in the small intestine.[5]

The growth performance of swine is greatly reduced at weaning.[1] This can be attributed to reduced feed intake[2] and a reduced ability of the GI tract to digest nutrients well.[5] This stage is also associated with increased susceptibility to gram negative bacterial infection, increased rate of diarrhea, and overall poorer health conditions.[3] These poor health conditions and reduced feed intake is due to depressed immune function.[2]

The most common approach for treating the outcomes of weaning stress is the use of antibiotics,[1] but this is becoming increasingly problematic due to antibiotic resistance and increased regulations on in feed antibiotics.[3] A few other alternatives either used or being studied include acidifiers, minerals (Cu and Zn), prebiotics, direct-fed microbials and yeast, nucleotides, and plant extracts (phytogenics).[6] They have varying ranges of efficacy, specific effects, and some have known modes of action while others do not.[6] For example, the mechanism of action of phytogenics is not fully understood,[7] with some studies showing positive effects of phytogenics on growth performance and overall health of weaned piglets and the others showing no effects.[6] As another dietary alternative, the use of low protein diets has been suggested for reducing the negative effects of weaning stress in nursery pigs.[8]

Low Protein Diets and Weaning Stress in Nursery Pigs

Costs of production continue to rise in the livestock industry. It is therefore important to investigate how the cost of production can be reduced and how to improve the production efficiency in our agricultural industries. Dietary protein is the most expensive component of the diet. The costs of dietary protein per piglet are $3.41 for 20 lb piglets, $3.10 for 22.5 lb piglets, and $2.86 for 25 lbs piglets.[4] The costs of dietary protein for a litter of 13 piglets whose average weight equals 22.5 lbs is therefore $40.30. The costs associated with dietary protein could potentially be reduced if piglets were fed low protein diets.[8] Finding ways to reduce the required amount of protein in diets without having negative influence on growth production could greatly reduce costs of production and improve protein use efficiency. Further, using low protein diets in weaning pigs could potentially help reduce the incidence of diarrhea that is common during this stage of production by decreasing the amount of substrate available for harmful bacteria in the gut.[8] Feeding low protein diets has been shown to decrease the proliferation of pathogenic bacteria and instead increase the population of healthy bacteria in the gut.[8] It also helps prevent the low pH in the large intestine, an occurrence which results in more favorable conditions for pathogenic bacteria.[9] Reducing the amount of crude protein in the diets of nursery pigs thereby helps relieve weaning stress by promoting beneficial bacterial growth and inhibiting that of pathogenic bacteria.

Effect of low protein diets on growth performance of nursery pigs

Along with beneficial effects of low protein diets on health of nursery pigs, there is, however, a down side to low protein diets in nursery pigs. When the dietary protein is severely decreased, it results in decrease in growth performance.[9] The decrease in performance due to feeding low protein diets is partly due to a deficiency of limiting amino acids. Slight reduction in dietary protein has shown negligible effects on growth performance.[8] However, when crude protein is reduced more than 4% unit, the growth performance is depressed despite the supplementation of limiting amino acids.[9]

Phytogenics and Weaning Stress in Nursery Pigs

"Phytogenic compounds comprise a wide range of plant-derived natural bioactive compounds" including essential oils and herbs.[10] There are numerous studies on phytogenics and their effects in various animal species and experimental settings that have proven that they have antimicrobial, antioxidative, and anti-inflammatory effects.[10] These effects make phytogenics promising alternatives to antibiotics as growth promoters and health maintenance compounds in the livestock industry. As mentioned earlier, alternatives to sub-therapeutic antibiotic use is needed due to the increasing concern over antibiotic resistant bacteria.

Phytogenics have the potential to improve protein use efficiency in swine, thereby they can reduce the production costs, without the concerns associated with increased antibiotic resistance.[2] This will appease consumers and regulatory agencies as well as provide a safer food supply. It is likely that, due to their many benefits to swine gut health, inclusion of phytogenics improve the body's ability to digest and absorb proteins. Some of the proposed mechanisms for the beneficial effects of phytogenics include increased feed intake, improved gut function, anti-oxidative effects, and antimicrobial effects.[7] The exact mechanisms underlying the effects of phytogenics on growth performance, however, are unknown. The proposed mechanisms have been shown to have varying degrees of accuracy in numerous studies, which used different combinations of phytogenic compounds from different sources and paired with different feed types.[11] In China, herbal feed additives are widely used, except in the large commercial industry, and in recent studies have proven to have beneficial effects on metabolism, immune response, intestinal health, and growth performance.[12] Challenges such as a lack of understanding of active ingredients and mechanisms along with quality control issues must be remedied before these additives can reach full effect in global commercial swine production.[12]

Effect of phytogenics on growth performance of nursery pigs

There are many studies on the various effects of phytogenic products, including essential oils and herbs, on growth performance and gut health in various stages of swine production. In a study on the effects of adding essential oils to the diets of weaned piglets, it was shown that some essential oils can improve the average daily gain and fecal scores, dry matter and crude protein digestibility, immune function, intestinal morphology, and raise the ratio of Lactobacillus: *E. coli* bacteria in the large intestine compared to a negative control diet and had similar effects as antibiotic diet.[1] In a study on the effects of anti-diarrheal herbs in weanling-growing piglets, it was shown that most of the herbs used improved average daily feed intake and average daily gain in weanling piglets, and all the herb included diets showed lower scores of diarrhea in piglets between days 2 and 6.[3] In another study on weanling piglets, the effects of a gel-based phytogenic feed supplement was shown not to affect average daily feed intake, to increase average daily gain, to improve gain to feed ratio through enhancement of dry matter and energy digestibility and to improve jejunum and ileum villi length.[2] All of these studies support the positive effects phytogenics on growth performance in weaned piglets.

Rationale, Hypothesis and Objectives

The most challenging stage of a pig's life is post-weaning.[5] This stage is associated with many health risks including diarrhea while antibiotics are no longer a viable option for prevention of illness and improving growth performance.[5] It is therefore imperative that new alternatives to sub-therapeutic use of antibiotics are found, which can improve health and performance during this stage of swine production. Low protein diets have the potential to help reduce weaning stress by decreasing the likelihood of the proliferation of harmful bacteria and increasing the likelihood of that of beneficial bacteria.[8] This strategy, however, can only be taken so far as decreasing protein too much will also hinder growth due to a lack of essential amino acids.[8] Therefore, finding ways to improve protein digestibility is important as it could potentially help with this problem. Another strategy to help reduce weaning stress is to use phytogenic additives.[6] These have been shown to have positive impacts on nursery pigs so long as the appropriate phytogenics with good quality are used.[12] Phytogenics also have the potential to improve protein absorption as some have been shown to improve overall nutrient digestablility.[2] To the best of our knowledge, there are no studies to assess the interaction of phytogenics and low protein diets on growth performance of nursery pigs.

We hypothesize that adding a phytogenic supplement to the water of nursery pigs fed with low protein diet would improve their growth performance. The objective of the current study was to assess the effects of a phytogenic supplement at three different doses on growth performance of weaned piglets fed with normal or low protein diets.

MATERIALS AND METHODS

Animals and Housing

A total of 48 weanling crossbred pigs (Duroc sire line and Large White X Landrace dam) barrows obtained from Sea Board Foods (Hennessey, Oklahoma) were used in this study. All pigs were weaned at 3-weeks of age. Upon arrival, all pigs were weighed (average weight of 5.8kg) and housed in groups.

The temperature of the facility was closely monitored and recorded daily. The temperature in week 1 was set at 31˚C and then was gradually decreased weekly (1 ˚C per week). Feed was provided in an open round trough to allow easy access to all pigs at the same time. Each pen was housed with three pigs during first two weeks of the trial (adaptation period), while pigs were housed individually upon starting the study. Each pen was equipped with a water nipple connected to a bucket with the appropriate amount of supplement to water ratio. After the trial started, feed was provided in stainless steel feeders that had an opening at the bottom to allow them access and an opening at the top to allow easy refilling. The floors were slatted plastic with a pit underneath with automatic disposal system for manure. All pigs were allowed *ad libitum* access to feed and water during both the adaptation period as well as during the trial itself.

Experimental Design

The animal care and use protocol was approved by the Animal Care and Use Committee of Oklahoma State University. After 2 weeks of adaptation, the pigs were weighed and individually housed. In 2 × 3 factorial design, all pigs were randomly assigned into 6 groups (9.1 ± 0.91 kg) receiving a combination of diets with 2 levels of protein (16% and 24% CP) and 3 levels of Herbanimal® supplement (0, 4, and 8 ml/L) in water (8 pigs/group) for 4 weeks. The groups were included: 1) 24% CP + 0 ml/L Herbanimal® supplement (CON-NS), 2) 24% CP + 4 ml/L Herbanimal® supplement (CON-LS), 3) 24% CP + 8 ml/L Herbanimal® supplement (CON-HS), 4) 16% CP (low protein) + 0 ml/L Herbanimal® supplement (LP-NS), 5) 16% CP + 4 ml/L Herbanimal® supplement (LP-LS), 6) 16% CP + 8 ml/L Herbanimal® supplement (LP-HS).

Diets were formulated for three different phases including nursery phase 1 (N1), nursery phase 2 (N2) and nursery phase 3 (N3). N1, N2 and N3 diets was prepared to meet the requirements of weaned piglets according to the Swine Nutrient Requirements (NRC, 2012)[13]. N1 diet with 24% CP was fed during the first week of the adaptation period. N2 diet was provided to pigs for two weeks- one week of the adaption period and the first week of the experimental period. N3 was offered to all pigs during the remaining three weeks of the study. All diets were corn-soybean based and were isocaloric (Table 1). Low protein diets were formulated by reducing the soybean meal and increasing the corn to meet the identical metabolizable energy between dietary groups. All pigs had free access to water and feed.

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

	N2[2]		N3[3]	
	24%[2.1] CP	**16%[2.2] CP**	**24%[3.1] CP**	**16%[3.2] CP**
Ingredients[4] %				
Corn, yellow dent	45.04	64.72	57.06	73.49
Fish meal, menhaden	4.9	4.98	2.07	2.1
Soybean meal, 47.5% CP	36.22	13.94	38.39	19.95
Whey, dried	5.87	5.97	-	-
Corn starch	5.87	5.97	-	-
Dicalcium phosphate	0.76	0.85	0.99	1.15
Limestone	0.54	0.67	0.62	0.68
NSNG Nursery Vit. Premix[5]	0.19	0.19	0.18	0.2
Salt	0.49	0.50	0.57	0.52
Chromium oxide	0.50	0.50	0.50	0.50
NSNG Trace Mineral Premix[6]	0.07	0.06	0.07	0.08
Lysine, sulfate	-	1.12	-	0.88
DL-methionine	-	0.14	-	0.08
L-threonine	-	0.28	-	0.22
L-tryptophan	-	0.13	-	0.1
Calculated Chemical Composition[7]				
Dry Matter, %[8]	90.74	90.72	89.66	89.60
ME, kcal/kg	3375	3379	3309	3322
Crude Protein, %	24.71	17.03	24.26	17.83
Crude Fiber, %	2.31	2.01	2.64	2.39
Crude fat, %	3.36	3.46	3.57	3.66
Lysine, %	1.35	1.35	1.24	1.23
Threonine, %	0.84	0.8	0.8	0.76
Methionine, %	0.38	0.41	0.36	0.35
Tryptophan, %	0.27	0.28	0.27	0.27
Isoleucine, %	0.95	0.58	0.93	0.61
Valine, %	1.04	0.67	1.02	0.71
Leucine, %	1.85	1.31	1.86	1.41
Histidine, %	0.61	0.39	0,6	0.41
Arginine, %	1.51	0.85	1.51	0.97
Phenylalanine, %	1.06	0.66	1.07	0.73
Calcium, %	0.8	0.8	0.7	0.7
Available Phosphorous, %	0.4	0.39	0.32	0.33

[1] Composition of experimental diets % as fed basis
[2] Two weeks feeding from day 7 to 21
[2.1] Control group fed from day 7 to 21
[2.2] Low protein group fed from day 7 to 21
[3] Three weeks feeding from day 21 to 42
[3.1] Control group fed from day 21 to 42

[3.2]Low protein diet fed from day 21 to 42
[4] Corn, soybean meal, whey, corn starch, dicalcium phosphate, limestone and salt were obtained from Nutra Blend, LLC (Dallas, TX). DL-methionine (99%) (MetAMINO®) and lysine, sulfate (Biolys®) where obtained from Evonik (Kennesaw, GA). L-threonine (98.5%), L-tryptophan (98%) and L-valine (96.5%) were obtained from Ajinomoto (Overland Park, KS). L-isoleucine (98.5%) was obtained from Xinjiang Fufeng group through Evonik (Kennesaw, GA). L-alanine was obtained from Ajinomoto Health & Nutrition North America, Inc (Raleigh, NC). Chromium oxide was purchased from Fisher Scientific (Bartlesville, OK).
[5] NSNG Nursery Vit. Premix provided per kilogram of diet: vitamin A, 720,000 IU; vitamin D, 180,000 IU; vitamin E, 5,400 mg; vitamin K, 360 mg; vitamin B12, 3.15 mg; niacin, 6,750 mg; pantothenic acid, 2,250 mg; riboflavin, 675 mg; phytase, 61,200 FTU
[6] NSNG Trace Mineral Premix was purchased from Nutra Blend, LLC (Dallas, TX). Each kilogram of mix contained: copper sulfate, 11,000 ppm; iodine, 198 ppm; iron, 73,413 ppm; manganese, 22,046; selenium, 198 ppm; zinc, 73,413 ppm.
[7] Values were calculated using National Swine Nutrition Guide (NSNG; V 2.0)
[8] Values analyzed and adjusted for 89.6% DM

Growth Performance Measurements

The feed and water intake were measured daily, and the body weight was recorded weekly. Average daily gain (ADG) was determined by dividing the gained weight during the whole experiment for each pig to the duration of the study as follows: (final body weight-initial body weight)/28. Average daily feed intake (ADFI) was measured by dividing the summation of feed intake for each pig during the entire study to the length of study (28 days). The gain to feed ratio (G:F) was obtained by dividing the overall body weight gain to feed consumed for each pig.

Feed Sampling and Analysis

Feed samples (~500 g) were collected from each batch of feeds mixed and were stored at 4 ˚C until analysis. About 250g were sent to Servitech for analysis (Dodge City, Kansas). All samples were analyzed for CP[14], crude fat[15], calcium[16], phosphorous[16], and nitrogen[17].

Statistical Analysis

Overall growth performance data were analyzed using univariate GLM using SPSS (IMB SPSS Statistics version 23, Armonk, NY, USA). Weekly cumulative BW, FI, WI, and G:F ratio were analyzed using mixed procedure of SPSS with the effects of diet, time and the interaction of diet and time as fixed effects and pigs as random effect. Preplanned contrast analysis were used to separate the means of following groups: CON-NS v. CON-LS, CON-NS v. CON-HS, CON-NS v. LP-NS, CON-NS v. LP-LS, CON-NS v. LP-HS, CON-LS v. CON-HS, CON-LS v. LP-LS, CON-HS v. LP-HS, LP-NS v. LP-LS, LP-NS v. LP-HS, and LP-LS v. LP-HS. The data were expressed as means ± standard error of means (SEM). Data were considered significant at P-values < 0.05 and tendencies at $0.05 < P < 0.1$.

RESULTS

The effects of dietary protein levels and phytogenic supplementation on overall growth performance are shown in Table 2. Pigs fed CON-HS and LP-HS diets had a significantly different (*P* < 0.05) final BW and ADG and a tendency for difference (*P* < 0.1) in G:F ratio with CON-HS having the higher values for all. Pigs fed CON-NS and CON-HS diets had a significantly different final (*P* <0.05) BW and a tendency for a difference (*P* <0.1) in ADWI with CON-HS being highest in both. CON-LS and CON-HS had a significantly different ADWI (*P* < 0.05) with the CON-HS group consuming more water. CON-HS had the highest final BW, ADG, ADFI, ADWI, and G:F ratio. LP-HS had the lowest final BW and ADG compared to other groups. LP-HS and LP-LS had the lowest G:F ratio and were equivalent to each other. Numerically, LP-NS had the lowest ADFI and ADWI. Overall, the greatest difference in final BW was detected between CON-HS and LP-HS (*P* <0.05).

Table 2. Growth performance of nursery pigs fed two different levels of dietary protein and supplemented with three levels of Herbanimal®

	Diets[1]							
	CON-NS	**LP-NS**	**CON-LS**	**LP-LS**	**CON-HS**	**LP-HS**	SEM[2]	*P*-value
Initial BW, kg	8.82	9.05	9.11	8.77	9.63	8.77	0.17	0.740
Final BW, kg	25.34[b]	24.89	26.08	25.00	29.09[j]	23.25	0.56	0.085
ADG[3], kg/d	0.59[b]	0.57	0.60	0.58	0.70[j]	0.52	0.16	0.056
ADFI[3], kg/d	0.86	0.84	0.95	0.91	0.97	0.86	0.23	0.493
ADWI[3], L	3.09*	2.78	2.98[f]	3.54	4.17*[j]	2.98	0.17	0.223
G:F[3], kg/kg	0.69	0.64	0.70	0.61	0.72*	0.61*	0.02	0.249

[1]**CON-NS**: 24% crude protein, no supplement; **LP-NS**: 16% crude protein, no supplement; **CON-LS**: 24% crude protein, low supplement (4 ml/L); **LP-LS**: 16% crude protein, low supplement (4 ml/L); **CON-HS**: 24% crude protein, high supplement (8 ml/L); **LP-HS**: 16% crude protein, high supplement (8 ml/L)
[2]SEM: standard errors of means
[3]ADG: average daily gain; ADFI: average daily feed intake; ADWI: average daily water intake; G:F: gain:feed
[b]Within a row, values of groups CON-NS vs. CON-HS differ ($P < 0.05$); [f]CON-LS vs. CON-HS differ ($P < 0.05$); [j]CON-HS vs. LP-HS ($P < 0.05$)
*Within a row, values with a common superscript symbol tend to be different ($0.05 < P < 0.1$)

The effects of dietary protein levels and phytogenic supplementation on weekly growth performance are shown in Table 3. Pigs fed diets CON-LS compared with those fed LP-LS were significantly different ($P < 0.05$) for BWG during week 1 with CON-LS having the largest gain. Pigs fed diets LP-LS compared with those fed LP-HS were significantly different ($P < 0.05$) for BWG during weeks 1-3 with LP-HS having the highest BWG in week 1 and LP-LS had the highest in weeks 2 and 3. Pigs fed diets LP-NS compared with those fed LP-HS were significantly different ($P < 0.05$) in BWG during week 4 and tended to be different ($P < 0.1$) during week 2 with LP-NS having the largest values for all weeks. Compared with LP-HS, pigs fed CON-NS had higher BWG during week 3 ($P < 0.05$). Compared with those fed CON-HS, pigs fed diets CON-NS were significantly different (P <0.05) for BWG during week 4 and had a tendency for difference (P <0.1) during week 1 with CON-HS having the higher values in both weeks. Pigs fed CON-HS had larger BWG than LP-HS (P < 0.05) during weeks 2-4. Pigs fed diets CON-LS compared with those fed CON-HS were significantly different (P <0.05) in BWG during week 4 with CON-HS being larger. Overall, pigs fed CON-HS had the highest BWG during weeks 1 and 4 while CON-LS had the largest BWG in week 2 and CON-NS and LP-LS had equally high values for week 3. The lowest BWG for week 1 was for LP-LS and that for weeks 2-4 was LP-HS.

Pigs fed diets LP-LS and LP-HS were significantly different (P <0.05) in CFI during week 4. Compared with those fed LP-HS, pigs fed CON-HS were significantly different in CFI during week 4; CON-HS had the highest CFI values for all weeks except week 1. Relative to LP-HS, pigs fed diets LP-NS had a tendency for difference (P < 0.1) in CFI during weeks 1, 2, and 4 with LP-HS having the larger CFI values for weeks 1 and 2 and LP-NS having the largest value in week 4. Compared with CON-HS, pigs fed CON-NS had a tendency for difference (P < 0.1) in CFI during week 4 with CON-HS having the largest value. Overall, the largest values for CFI include LP-HS for week 1, CON-HS for weeks 2 and 4, and LP-LS for week 3. Pigs fed LP-NS had the lowest CFI value during weeks 1-3 and LP-HS had the lowest for week 4.

Pigs fed diets CON-LS compared with those fed CON-HS were significantly different (P < 0.05) in CWI during week 3 with CON-HS having the largest value. Compared to LP-LS, pigs fed diets CON-LS were significantly different (P < 0.05) in CWI during week 4. Compared with those fed LP-HS, pigs fed diets LP-LS were significantly different (P < 0.05) in CWI during week 4 with LP-LS as the larger value. Relative to

CON-HS, pigs fed diets CON-NS had a tendency for difference (*P* <0.1) in CWI during week 1; CON-HS had the larger value. In comparison with LP-NS, pigs fed CON-NS had a tendency for difference (*P* < 0.1) in CWI during week 2; CON-NS pigs had highest water consumption in that week. Compared with those fed LP-HS, pigs fed LP-NS had a tendency for difference (*P* <0.1) in CWI during week 2 with LP-HS having the larger value. Pigs fed diets CON-HS compared with those fed LP-HS had a tendency for difference (*P* <0.05) in CWI during week 3; CON-HS pigs consumed the larger quantity of water. Pigs fed diets LP-NS compared with those fed LP-LS had a tendency for difference (*P* <0.05) in CWI during week 4 with LP-LS having the larger value. Overall, CON-HS had the largest CWI values for weeks 1-3 while LP-LS had the largest for weeks 4. The lowest values for CWI are observed in diets LP-LS on week 1, LP-NS on week 2, CON-LS on week 3, and LP-HS on week 4.

Pigs fed diets CON-NS compared with those fed LP-HS were significantly different (*P* < 0.05) in G:F ratio during weeks 2 and 3 with CON-NS having the larger value in both weeks. Compared with those fed LP-HS, pigs fed LP-NS diets were significantly different (*P* < 0.05) in G:F ratio during weeks 1-3; while LP-HS had the largest ratio during week 1, LP-NS had the larger ratios for weeks 2 and 3. In comparison with those fed LP-LS, pigs fed CON-LS were significantly different (*P* < 0.05) in G:F ratio during week 1 with CON-LS having the larger ratio. LP-HS had the larger G:F ratio during week 1, but LP-LS had the higher value during week 2. Relative to CON-HS, pigs fed CON-LS had a tendency for difference (*P* <0.1) in G:F ratio during week 4 as did pigs fed diets CON-HS compared with those fed LP-HS. CON-HS had the larger G:F ratio in both comparisons. Overall, CON-HS had the largest G:F ratios during weeks 1 and 4 while LP-LS had the highest G:F ratio in week 2 and LP-NS had the largest value in week 3. LP-NS and LP-LS shared the lowest value for the G:F ratio for week 1 as do CON-LS and LP-LS for week 4. LP-HS has the lowest G:F ratio for weeks 2-3.

Table 3. Weekly growth performance of nursery pigs fed two different levels of protein and supplemented with three levels of Herbanimal®.

	Diets[1]							
	CON-NS	**LP-NS**	**CON-LS**	**LP-LS**	**CON-HS**	**LP-HS**	SEM[2]	*P-value*
BWG [3], kg								
Wk 1	1.98*	1.36	2.32^{k}	1.24^{i}	2.97*	2.77	0.17	0.00
Wk 2	3.96	3.34*	4.24	4.13^{i}	4.20^{j}	3.22*	0.14	0.10
Wk 3	5.09^{e}	4.92*	4.75	5.09^{i}	4.91^{j}	3.73*	0.18	0.24
Wk 4	5.48^{b}	6.22^{h}	5.66^{f}	5.77	7.37^{j}	4.75	0.22	0.01
CFI [3], kg								
Wk1	3.35	2.97*	3.63	3.10	3.76	3.82*	0.14	0.40
Wk 2	5.11	4.61*	5.83	5.33	6.17	5.80*	0.19	0.17
Wk 3	7.30	6.79	7.72	7.83	7.38	6.85	0.22	0.68
Wk 4	8.25$^{\#}$	9.19*	9.48	9.30^{i}	9.82$^{\#j}$	7.57*	0.27	0.12
CWI [3], L								
Wk 1	11.81*	12.00	14.88	10.94	18.21*	12.50	0.97	0.30
Wk 2	22.06*	12.88$^{*\#}$	21.69	20.25	28.14	22.12$^{\#}$	1.60	0.17
Wk 3	24.69	25.50	21.50^{f}	29.31	34.07*	23.81*	1.64	0.31
Wk 4	28.06	27.38*	25.31^{k}	38.62^{*i}	36.21	25.00	1.96	0.19
G:F [3], kg/kg								
Wk 1	0.62	0.40^{h}	0.64^{k}	0.40^{i}	0.77	0.74	0.05	0.01
Wk 2	0.78^{e}	0.78^{h}	0.74	0.79^{i}	0.69	0.56	0.02	0.05
Wk 3	0.71^{e}	0.74^{h}	0.66	0.66	0.67	0.53	0.02	0.19
Wk 4	0.68	0.68	0.62*	0.62	0.75$^{*\#}$	0.63$^{\#}$	0.01	0.32

[1]CON-NS: 24% crude protein, no supplement; LP-NS: 16% crude protein, no supplement; CON-LS: 24% crude protein, low supplement (4mL/L); LP-LS: 16% crude protein, low supplement (4mL/L); CON-HS: 24% crude protein, high supplement (8mL/L); LP-HS: 16% crude protein, high supplement (8mL/L)
[2] SEM: Standard error of mean
[3]BWG: body weight gain; CFI: cumulative feed intake; CWI: cumulative water intake; G:F: gain:feed.
^{b}Within a row, values of CON-NS and CON-HS differ ($P < 0.05$); ^{e} CON-NS and LP-HS differ ($P < 0.05$); ^{f}CON-LS vs. CON-HS differ ($P < 0.05$); ^{h}LP-NS vs. LP-HS differ ($P < 0.05$); ^{i}LP-LS vs. LP-HS differ ($P < 0.05$); ^{j} CON-HS vs. LP-HS ($P <0.05$); ^{k} CON-LS vs. LP-LS ($P <0.05$)
$^{*\#}$Within a row, values with a common superscript symbol tend to be different ($0.05<P < 0.1$)

The trend of body weight, averaged by dietary group, throughout the trial with the data points being days 0, 7, 14, 21, and 28 is shown in Figure 1. Over most of the trial period, the diets follow similar trends with CON-HS being slightly higher than the others for the whole trial and LP-NS and LP-LS remaining below most of the other dietary groups until after day 21. The data was not statistically significant for any day other than day 28. On this day, groups CON-NS and CON-HS were significantly different ($P < 0.05$) with CON-HS having the greatest BW. The groups CON-HS and LP-HS were also significantly different ($P < 0.05$) on day 28 with CON-HS having the largest BW. Overall, Figure 1. shows diets CON-HS pigs as having the largest BW throughout the trial period, excluding day 0. The graph also shows LP-HS as having the lowest body weight at the end of the trial with LP-NS and LP-LS having distinctly surpassed it after day 21.

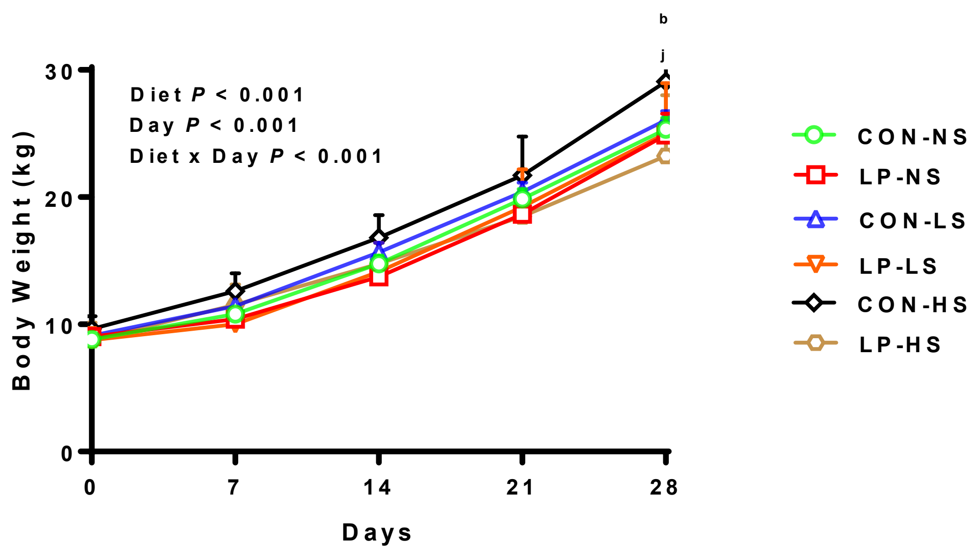

Figure 1. Body weight of nursery pigs fed with low protein diets.
CON-NS: 24% crude protein + no supplement; **LP-NS**: 16% crude protein + no supplement; **CON-LS**: 24% crude protein + low supplement (4mL/L); **LP-LS**: 16% crude protein + low supplement (4mL/L); **CON-HS**: 24% crude protein + high supplement (8mL/L); **LP-HS**: 16% crude protein + high supplement (8mL/L).
Superscripts show contrasts between individual treatments on specific days and represent the following: [b]CON-NS vs. CON-HS differ ($P < 0.05$); [j] CON-HS vs. LP-HS ($P < 0.05$)

The overall feed intake of nursery pigs throughout the trial with data points at days 1, 3, 5, 7, 9, 11, 12, 14, 16, 18, 20, 21, 23, 24, 26, and 27 is shown in Figure 2. On day 1, there was a significant difference ($P < 0.05$) when comparing FI of CON-HS and LP-HS with CON-HS having the lower value. When comparing these two groups on day 26, there is a significant difference ($P < 0.05$) between them with CON-HS having higher value of FI. On day 5, when comparing the groups CON-NS with CON-HS, CON-HS has a significantly higher ($P < 0.05$) FI value than CON-NS as did LP-LS when compared with LP-HS on the same day. LP-HS also had significantly higher FI ($P < 0.05$) compared to LP-LS on days 7, 9, 11, and 12. On day 5, there were significant differences ($P < 0.05$) between CON-NS compared with LP-HS and between LP-NS and LP-HS. LP-HS had the highest value in both cases. The LP-NS and LP-HS also had significantly different FI ($P < 0.05$) on days 12, 22, and 26 during which LP-HS had a higher FI on day 12 and a lower FI on days 23 and 26. On day 7 and 11, compared with LP-LS, CON-LS had higher FI ($P < 0.05$).

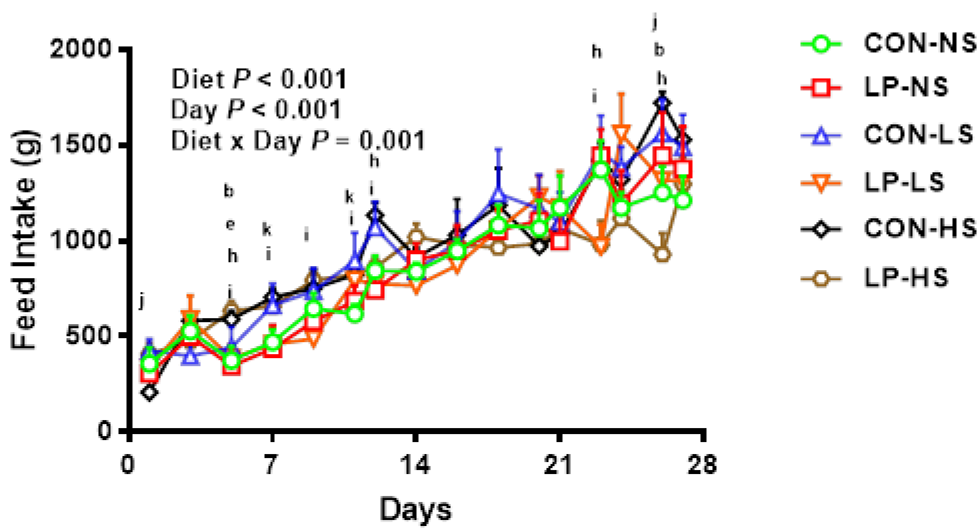

Figure 2. Feed intake of nursery pigs fed with low protein diets containing three levels of phytogenic supplement.

CON-NS: 24% crude protein + no supplement; **LP-NS**: 16% crude protein + no supplement; **CON-LS**: 24% crude protein + low supplement (4mL/L); **LP-LS**: 16% crude protein + low supplement (4mL/L); **CON-HS**: 24% crude protein + high supplement (8mL/L); **LP-HS**: 16% crude protein + high supplement (8mL/L). Superscripts show contrasts between individual treatments on specific days and represent the following: [b]CON-NS and CON-HS differ ($P < 0.05$), [e]CON-NS and LP-HS differ ($P < 0.05$), [h]LP-NS and

LP-HS differ ($P < 0.05$), [i]LP-LS vs. LP-HS differ ($P < 0.05$); [j] CON-HS vs. LP-HS ($P <0.05$); [k] CON-LS vs. LP-LS ($P <0.05$)

DISCUSSION

Weaning is highly stressful period for pigs, and nursery pigs often face negative effects such as decrease in FI,[2] higher risk for disease,[3] and depressed growth performance.[1] Antibiotics have been used for years to remedy this problem but due to the rising problem of antibiotic resistant bacteria alternatives are being sought out. There are some studies that have tested the effectiveness of phytogenics as replacements for sub-therapeutic levels of antibiotics. Some studies have supported the use of phytogenics and others have proven them ineffective. For instance, in a study on the effects of various essential oils on growth performance in weaned pigs, three of the four supplements resulted in improved ADG and G:F ratios as compared to the other essential oil diet and the control diet.[3] This is in part due to the fact that different types of phytogenics relate directly to their effectiveness in swine production as well as the fact that these phytogenics may have originated from different sources as that can greatly affect their quality and effectiveness.[12] Another study showed that gel-based phytogenic supplements have the potential to improve growth performance even while reducing the ADFI.[2] While another study on the effects of adding one essential oil supplement showed similar growth performance compared to the positive control and improved performance as compared to the negative control.[1] Low protein diets have been shown to reduce the negative effects of weaning stress but can cause decreases in growth performance if dietary protein is reduced too much.[9]

Our study looked at the combination of phytogenic with low protein diets as solution to weaning stress. Our results showed that there was a slight decrease in final BW of the low protein diet fed pigs with herbal supplemented at higher dose compared to those fed with low protein diet with no supplement/lower dose supplement, although this difference was insignificant (*P* > 0.05). This indicates that the Herbanimal® supplement was not effective in improving the growth performance of nursery pigs fed with low protein diets. The highest BW and ADG was seen in pigs fed with normal crude protein diet supplemented with high dose of Herbanimal®. This result lines up with the gel-based phytogenic study as in that study, the positive effects of adding phytogenics to a normal basal diet was reported, although our result showed a lesser effect of the supplement on G:F compared to that study.[2] This suggests that while the phytogenic supplement we tested was not effective in improving growth performance of low protein diets, it was effective in improving growth performance of nursery pigs fed normal diets and given no antibiotics. Our results differed slightly from the study that compared the effect of one essential oil supplement with a group fed sub-therapeutic levels of antibiotics (positive control) and another given no supplements of any kind (negative control).[1] In their study, they showed improvements between their negative control and their essential oil supplemented group whereas in our study, there was a lower final BW and ADG in the LP-HS compared to the LP-NS diet, although there was a numerical difference between the LP-LS and LP-NS in our study with the LP-LS diet having slightly higher ADG and final BW. Similar to previously mentioned study, supplementation of normal protein diets with phytogenics produced positive effects on final BW and ADG. Comparing dietary protein levels with this study is relevant in that their crude protein levels were approximately 20% which is 4% away from both of our protein level groups. It may, however be more relevant to compare the 24% crude protein level data with theirs in that according to one study, reducing the crude protein levels by more than 4% of the recommended NRC[13] levels will have negative effects on growth performance despite the addition of limiting amino acids.[9]

There is a greater significance (P <0.05) and tendency toward significance (0.05< P <0.1) in the differences between diets in the weekly data than in the overall data. This is most prominent in BWG and G:F ratio. This means that there are more significant differences (P <0.05) in growth performances between the groups on a week-to-week basis than for all 4 weeks of the trial. The weekly data in Table 3. also shows that for all weeks except week 3, the group with the highest CFI were high supplement groups. On week 3, the group with the highest CFI is a low supplement group. This suggests that the supplement increases the desire to eat which could potentially be one reason why the CON-HS group showed the greatest growth performance, as it is a high supplement group with a normal level of dietary protein.

Overall, our data suggests that Herbanimal® supplementation in water had positive effects on growth performance of pigs fed with normal protein diets, but it did not improve the growth performance of pigs fed with slightly low protein diets.

CONCLUSION

In conclusion, our hypothesis that adding a phytogenic supplement to the water of nursery pigs fed with low protein diet would improve their growth performance was disproven with showing no positive effect of Herbanimal® on growth performance of pigs fed with low protein diets. Our results did, however, proved that Herbanimal® can be effective in improving growth performance in nursery pigs when they are fed with normal crude protein levels suggestive of it is a potential to be applied as alternative for sub-therapeutic antibiotics. Future studies should directly compare the Herbanimal® with antibiotics on their effects on growth performance of nursery pigs. There are also needs for studies testing the effects of other phytogenic supplements on growth performance of nursery pigs fed low protein diets. As discussed above, different phytogenics may not result in similar effects and there may be one that can prove our hypothesis and thus reduce the costs of swine production.

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VITA

Jessie Barnes

Candidate for the Degree of Honors

Master of Science/Arts

Thesis: EFFECT OF WATER-BASED PHYTOGENIC SUPPLEMENT ON GROWTH PERFORMANCE OF NURSERY PIGS FED LOW PROTEIN DIETS

Major Field: Animal Science

Biographical:

I am from Edmond, OK and was born on May 15th, 1998. I am currently working seasonally at VCA Kickingbird Animal Hospital in Edmond, OK and previously worked at White Oaks Veterinary Clinic. I graduated from high school with a 4.0 after having taken Pre-AP and AP classes, passing all but one test with a 4 or higher and passing my first one with a 3. Since being in college, I have earned Early Admissions to OSU-CVHS and am pursing a bachelors degree through the 3 plus 1 program. I have also made it through Survey Organic Chemistry and Survey of Biochemistry with a 4.0 despite battling depression.

Education:

Completed the requirements for a high school diploma from Edmond Memorial High School in May 2016.

Will complete the requirements for the Bachelor of Science/Arts in your Animal Science at Oklahoma State University Stillwater, OK in May 2020.

Experience:

Professional Memberships: The Honors Society of Phi Kappa Phi, Golden Key International Honors Society