

Title: Effect of Adding Peptiva in Replacing Fish Meal on Growth Performance in Nursery Pigs.

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Introduction

In pigs, weaning stress includes change in diet form and social interaction, such as competing for dominance order and isolation from the dam, which often results in reduced intake and disruption in intestinal barrier integrity, which allows opportunistic pathogenic bacteria to become dominant in the commensal microfloral community and leads to post weaning diarrhea, growth retardation, and even death. Therefore, early nursery diets typically are formulated using ingredients that are not only highly bioavailable but also palatable to serve as means to stimulate intake in order to provide sufficient amounts of nutrients to increase recovery rate and attenuate the negative impact of the weanling process.

Porcine derived Hydrolysate peptides have been suggested to increase voluntary feed consumption in nursery pigs, to a level comparable to pigs fed diets containing whey (Solà-Oriol et al., 2011) but was less preferred when compared to pigs fed lactose (Figuerola et al., 2016). Fish derived hydrolysate peptides, Peptiva (Vitech Bio-Chem, Corp, CA), was reported to improve intake, which is in agreement with finding by Norgaard et al., 2012 who demonstrated that Peptiva effectively restored weight gain when compared to pigs fed spray dried plasma protein (SDPP) in trials that were conducted at Virginia tech and the University of Georgia using diets where AA were balanced to meet the ideal protein suggested requirements. In addition, economic returns were higher with Peptiva than SDPP diets. In a field trial, It was suggested that the combination of Peptiva and probiotics/prebiotic could potentially have a synergetic effect.

A study conducted at the university of Arkansas with “Peptiva SEW” suggested that 3% Peptiva+ZnO can restored growth performance when compare to those fed SDPP+ZnO diets. The new product “Peptiva Swine” has been reported from field trials to have greater potential to improve performance in swine than “Peptiva SEW”.

We propose to evaluate the optimum level of “Peptiva Swine” in nursery diets with a titration study, and compare results with or without ZnO

Specific Objective:

- 1) Determine the effect of Peptiva Swine in replacing fish meal on growth performance, complete blood cell counts, blood urea nitrogen and microbiome community in nursery pigs fed diets devoid of ZnO.
- 2) Determine the effect of Peptiva Swine in replacing fish meal on growth performance, complete blood cell counts, blood urea nitrogen and microbiome community in nursery pigs fed diets devoid of ZnO.
- 3) Determine optimum level of Peptiva Swine in nursery diets.

Materials and Methods

Nursery phase:

A total of 288 PIC C-29 X PIC 380 pigs, at approximately 21 days of age from the University of Arkansas Animal Science Swine Research Farm, were used for the study.

Allotment to Treatments:

The pigs were individually weighed and sorted at weaning. To avoid the confounding effect of initial weight, the pigs were assigned to 6 blocks by weight as determined by the experimental facility (6 blocks of 48 pigs per block). There were a total of 6 replicates per treatment in each phase, with pigs housed 6 pigs/pen. An attempt will be made to balance sex within block such that each treatment was represented by equal numbers of each sex within block. Pigs remained in the same pens throughout the experiment.

Treatment Arrangement:

All pigs were fed a common phase 1 diet for 14 days. There were 8 treatments fed over two different diet regimes, and pigs remained on the same dietary treatment throughout the entire study period.

Table 1. Phase 1: 6-8 kg (14 d) – common diets

Table 2. Phase 2: 8-14 kg (14 d) – 1 of 8 experimental diets

Table 3. Phase 3: 14-22 kg (14 d) – 1 of 8 experimental diets

Phase 1, 2 and 3 Treatments:

Treatment 1 -- Positive control moderately complex diet with fish meal + ZnO.

Treatment 2 -- Negative Control devoid of fish meal + ZnO.

Treatment 3 -- NC + 0.05% Peptiva Swine.

Treatment 4 -- NC + 0.25 % Peptiva Swine.

Treatment 5 -- NC + 0.50% Peptiva Swine

Treatment 6 – NC + 0.05% Peptiva Swine + ZnO

Treatment 7 – NC + 0.25% Peptiva Swine + ZnO

Treatment 8 – NC + 0.50% Peptiva Swine + ZnO

Diets Formulation, Requirements, Mixing and Sampling:

Dietary formulation was provided by University of Arkansas. Diets for the study are presented in Tables 1, 2, and 3 for nursery phases 1, 2 and 3, respectively.

Housing:

Pigs were housed in a nursery, and each 4.90 × 3.95 foot pen is equipped with a two-hole feeder and one waterer for ad libitum access to diets and water. Ambient temperature was set at 85° F upon pig arrival, and decreased by two degrees per week to approximately 75° F at the end of nursery.

Measurements:

Individual pig weights and pen feed intake were collected in order to calculate average daily gain, feed intake, and gain-to-feed ratio by phase. Blood samples were collected on d 14, 28 and 42 from a median BW pig of each pen from body weight blocks 1, 2, 5, 6 while two median BW pigs of each pen from body weight blocks 3, and 4 (to have a total of 8 replicates per treatment) into K2EDTA tube

and 1 mL of whole blood was aspirated into micro centrifuge tubes for complete blood cells count using Hemavet instrument. Afterward, remaining samples were centrifuged and plasma was aspirated into 5 mL sample storage tubes, and stored at -20°C for BUN determination.

Rectal swabs samples were collected from the same pigs of those used for blood collection on d 14, 28 and 42 to determine microbiome population via Miseq technique. Data was then assess by mothur program and sequences were aligned with Silva reference database, and taxonomy was classified against 16S rRNA reference (RDP) database.

Feed samples

A feed sample was obtained for each batch of feed mixed and stored in the freezer until study completion. A Composite feed samples for each phase was mailed to:

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Animal care:

The pigs in this study were cared for according to typical commercial management procedures. This experiment was carried out in accordance with the Animal Care Protocol # 18037 for swine experiments issued by the University of Arkansas Animal Care Committee. Any animal suffering from minor illness was reported to the Study Director and treated. All medical treatments were recorded. Any animal that dies or becomes ill was weighed and removed from the study. An animal removal form was completed detailing the reason for removal, date, time and animal disposition.

Data analysis:

Performance and CBC data were analyzed by PROC Mixed procedure of SAS (SAS Institute, Inc., Cary, NC). Dietary treatment were the lone fixed effect, and blocks based on initial BW was the random effect, and will pen serve as the experimental unit for ANOVA. Peptiva Swine inclusion rates were used in the IML Procedure of SAS to generate coefficients, which then were incorporated in orthogonal contrast analysis for treatment 2, 3, 4 and 5 as well as treatment 2, 6, 7, and 8.

Results: Overall results of this study were very good although we experienced a larger number of removals than we typically experience due to individual pig weight loss or

death. A total of 11 pigs (3.8%) were removed. This could be due to the fact that no antibiotic or ZnO were included in the diet during phase 1 (d 0 to 11) when pigs were fed a common moderately complex phase 1 diet prior to study initiation. It is also interesting to note that 9 of the 11 pigs removed (82%) were either fed the negative control diet or the lowest level of Peptiva swine during phase 2 and 3. No pigs were removed when diets contained either 0.25 or 0.50% Peptiva swine. Although this is interesting, there are not sufficient animal numbers to determine statistical differences.

Gain during phase 2 and 3 (Table 4) was similar in pigs fed the positive control, high protein diet (HP) or the negative control, low protein diet (Figure 1, Figure 2). However, during the overall study (d11-40) ADG was greater in pigs fed the HP diet (Figure 3). ADG increased with increasing dietary Peptiva (from 0 in the negative control to 0.50% Peptiva) in pigs fed diets devoid of ZnO or diets containing ZnO although the magnitude of response was greater in pigs fed increasing Peptiva in combination with ZnO in phase 1 (d11-21) phase 2 (d21-40) or overall study (Table 6; Figures 2, 3 and 4, respectively; Peptiva X ZnO, Linear effect, $P < 0.01$ in phase 1 and Peptiva X ZnO, Quadratic effect, $P < 0.05$ in phase 2 and 3).

Body weight between pigs fed the HP and LP diets were similar at the end of phase 2 but by study completion (d40), pigs fed the HP diet were heavier (Table 4). As might be expected by ADG, BW increased with increasing Peptiva with the response greater in pigs fed ZnO (Table 6). This resulted in a Peptiva X ZnO, Linear effect, $P < 0.05$ at the end of phase 2 and a Peptiva X ZnO, Quadratic effect, $P < 0.05$ at study completion (d40).

ADFI was similar between pigs fed the HP or LP diets (Table 5). ADFI tended to increase in pigs fed diets with increasing Peptiva and the Peptiva effect appeared to be greater in pigs fed diets with ZnO, although differences were not significant (Figures 4 and 5). ADFI did, however, tend to increase in pigs fed diets without ZnO during phase 2 (Table 7, Figure 4, d21-40, $P < 0.10$). During other time periods ADFI was similar ($P > 0.13$).

G:F was similar between pigs fed the HP or LP diets (Table 5). During phase 1 (d11-21), pigs increasing the level of Peptiva tended to increase G:F in pigs fed diets without ZnO ($P < .10$) and increased G:F in pigs fed diets with ZnO ($P < 0,001$). Although this followed the pattern of increasing Peptiva response in pigs fed ZnO observed with ADG, the Peptiva X ZnO interaction for G:F only approached significance (Table 7, $P < 0.13$).

This study demonstrates that Peptiva effectively enhances gain in pigs fed a low protein diet devoid of Plasma protein and that this enhanced performance is greater in combination with feeding ZnO.

Table 1. Nursery phase 1 diets composition.

University of Arkansas	PC
Trial: 2017 Peptiva Swine	8-12
Ingredients	%
Corn, Yellow Dent	39.77
Soybean meal, 48%, high	22.500
Corn DDGS, >6 and <9%	0.000
Poultry Fat	3.000
Monocalcium P	0.650
Limestone	0.450
Salt	0.250
L-Lysine	0.210
DL-Methionine	0.165
L-Threonine	0.049
L-Tryptophan	0.028
ZnO	0.000
Copper Sulfate	0.000
Trace Mineral Premix (NE	0.150
Vitamin Premix (NB-6508	0.250
Plasma (AP-920)	3.000
Fish Meal, Menhaden	6.000
Milk, Whey Powder	20.000
Peptiva Swine	0.000
Ethoxyquin (Quinguard)	0.030
L-Valine	0.000
Milk, Lactose	3.500
Ronozyme HiPhos 2700 (0.003
Total	100.0
Calculate	
NSNG ME (kcal/kg)	3487
CP (%)	22.807
SID Lysine (%)	1.462
Total P (%)	0.765
Available P (%)	0.552
Aval. P (%) with phytase	0.581
Ca (%)	0.853
gSID Lysine/Mcal ME	4.19
SID Met:Lys	34.89
SID M+C:Lys	58.07
SID Thr:Lys	60.09
SID Trp:Lys	20.05
SID Ile:Lys	58.98
SID Val:Lys	66.92
SID Leu:Lys	118.48
SID His:Lys	37.45
SID Arg:Lys	85.12
SID Phe:Lys	63.74
SID Tyr:Lys	45.06
SID Phe+Tyr:Lys	108.72

Table 2. Nursery phase 2 diets composition.

University d	PC	NC	NC+0	NC+0	NC+0	NC+0	NC+0	NC+0
Trial: 2017	16-25	16-25	16-25	16-25	16-25	16-25	16-25	16-25
Ingredients	%	%	%	%	%	%	%	%
Corn, Yellow	56.38	59.06	59.01	58.81	58.56	58.69	58.49	58.24
Soybean meal	27.60	27.60	27.60	27.60	27.60	27.60	27.60	27.60
Corn DDG	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Poultry Fat	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium	0.54	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Limestone	0.93	1.16	1.16	1.16	1.16	1.16	1.16	1.16
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
DL-Methionine	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Threonine	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
L-Tryptophan	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
ZnO	0.32	0.00	0.00	0.00	0.00	0.32	0.32	0.32
Trace Minerals	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Plasma (All)	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Fish Meal	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Peptiva Sw	0.00	0.00	0.05	0.25	0.50	0.05	0.25	0.50
Ethoxyquin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L-Valine	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Ronozyme	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Total	100	100	100	100	100	100	100	100
Calculate								
NSNG ME	3437	3427	3427	3425	3424	3416	3415	3413
CP (%)	23.0	21.3	21.4	21.4	21.5	21.3	21.4	21.5
SID Lysine	1.42	1.29	1.29	1.29	1.30	1.29	1.29	1.30
Total P (%)	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Available P	0.35	0.35	0.35	0.35	0.36	0.35	0.35	0.36
Aval. P (%)	0.38	0.38	0.38	0.38	0.39	0.38	0.38	0.39
Ca (%)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
gSID Lysin	4.13	3.76	3.76	3.78	3.80	3.77	3.79	3.81
SID Met:Ly	36.0	36.1	36.1	36.1	36.0	36.1	36.1	36.0
SID M+C:L	58.0	59.6	59.6	59.6	59.5	59.6	59.5	59.4
SID Thr:Lys	60.0	61.2	61.2	61.2	61.1	61.2	61.1	61.1
SID Trp:Lys	19.1	19.7	19.7	19.7	19.7	19.7	19.7	19.7
SID Ile:Lys	56.7	57.4	57.4	57.3	57.3	57.3	57.3	57.3
SID Val:Lys	67.0	68.0	68.0	68.0	68.0	68.0	67.9	67.9
SID Leu:Lys	121.1	125.3	125.2	125.1	124.9	125.1	124.9	124.7
SID His:Lys	38.3	38.8	38.7	38.7	38.7	38.7	38.7	38.7

Table 3. Nursery phase 3 diets composition.

University d	PC	NC	NC+0.0	NC+0.1	NC+0.2	NC+0.3	NC+0.4	NC+0.5
Trial: 2017	25-50	25-50	25-50	25-50	25-50	25-50	25-50	25-50
Ingredients	%	%	%	%	%	%	%	%
Corn, Yellow	50.29	54.79	54.74	54.54	54.29	54.42	54.22	53.97
Soybean meal	28.20	23.96	23.96	23.96	23.96	23.96	23.96	23.96
Corn DDG	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Poultry Fat	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium	0.40	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Limestone	1.10	1.11	1.11	1.11	1.11	1.11	1.11	1.11
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Threonine	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
L-Tryptophan	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
ZnO	0.32	0.00	0.00	0.00	0.00	0.32	0.32	0.32
Trace Minerals	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Peptiva Sw	0.00	0.00	0.05	0.25	0.50	0.05	0.25	0.50
Ethoxyquin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ronozyme	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Total	100	100	100	100	100	100	100	100
Calculate								
NSNG ME	3426	3439	3439	3437	3435	3428	3426	3425
CP (%)	22.4	20.8	20.8	20.9	21.0	20.8	20.9	21.0
SID Lysine	1.28	1.18	1.18	1.19	1.19	1.18	1.19	1.19
Total P (%)	0.51	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Available P	0.25	0.25	0.25	0.25	0.26	0.25	0.25	0.26
Aval. P (%)	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Ca (%)	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
gSID Lysine	3.75	3.43	3.44	3.45	3.47	3.45	3.46	3.48
SID Met:Lys	35.5	37.1	37.0	37.0	36.9	37.0	37.0	36.9
SID M+C:L	58.1	60.1	60.1	60.0	59.9	60.0	59.9	59.8
SID Thr:Lys	60.0	60.4	60.4	60.4	60.3	60.4	60.4	60.3
SID Trp:Lys	19.0	18.7	18.7	18.7	18.7	18.7	18.7	18.7
SID Ile:Lys	60.2	59.5	59.5	59.5	59.4	59.5	59.4	59.4
SID Val:Lys	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0
SID Leu:Lys	134.5	138.0	138.0	137.7	137.5	137.8	137.6	137.3
SID His:Lys	39.8	39.9	39.9	39.9	39.9	39.9	39.9	39.8

Table 4. Effect of Peptiva Swine on ADG and BW in nursery pigs fed high or low protein diets with or without ZnO¹

	HP	LP	Peptiva Swine						SEM	P - Value
	0%	0%	0.05%	0.25%	0.50%	0.05%	0.25%	0.50%		
ADG, kg/d										
d 11-21	0.274 ^{ab}	0.253 ^a	0.235 ^a	0.224 ^a	0.272 ^{ab}	0.234 ^a	0.307 ^b	0.360 ^c	0.018	<0.0001
d 21-40	0.609 ^b	0.539 ^{ab}	0.562 ^b	0.505 ^a	0.543 ^a	0.585 ^b	0.604 ^b	0.592 ^b	0.024	0.0012
d 11-40	0.500 ^c	0.441 ^{ab}	0.453 ^b	0.407 ^a	0.452 ^b	0.477 ^{bc}	0.502 ^c	0.514 ^c	0.018	<0.0001
BW, kg										
d 11	5.60	5.73	5.65	5.64	5.69	5.44	5.63	5.60	0.45	0.1771
d 21	8.34 ^{bc}	8.32 ^{abc}	8.08 ^{ab}	7.88 ^{ab}	8.41 ^{bc}	7.77 ^a	8.70 ^{cd}	9.20 ^d	0.51	0.0006
d 40	20.22 ^c	18.57 ^b	18.90 ^b	17.45 ^a	18.83 ^b	19.38 ^{bc}	20.21 ^c	20.51 ^c	0.92	<0.0001

¹ADG d 0 to 11 and feed efficiency d 0 to 11 were used as covariant for BW and ADG analysis.

a, b, c. Means with a different superscript differ. P < 0.01.

Table 5. Effect of Peptiva Swine on ADFI and G:F in nursery pigs fed high or low protein diets with or without ZnO¹

	HP	LP	Peptiva Swine						SEM	P -Value
	0%	0%	0.05%	0.25%	0.50%	0.05%	0.25%	0.50%		
ADFI, kg/d										
d 11-21	0.315	0.310	0.292	0.301	0.310	0.299	0.345	0.336	0.018	0.3173
d 21-40	0.682 ^d	0.643 ^{de}	0.719 ^e	0.626 ^d	0.663 ^d	0.724 ^{ef}	0.746 ^f	0.770 ^f	0.037	0.0288
d 11-40	0.555 ^{de}	0.528 ^d	0.572 ^{de}	0.514 ^d	0.541 ^d	0.577 ^{de}	0.608 ^e	0.620 ^e	0.027	0.0338
G:F										
d 11-21	0.883 ^{ab}	0.797 ^{ab}	0.772 ^{ab}	0.749 ^a	0.893 ^b	0.783 ^{ab}	0.890 ^b	1.056 ^c	0.047	0.0011
d 21-40	0.864	0.840	0.791	0.808	0.827	0.798	0.818	0.772	0.027	0.3367
d 11-40	0.867	0.832	0.786	0.796	0.837	0.796	0.833	0.827	0.024	0.2666

¹ADFI d 0 to 11 and feed efficiency d 0 to 11 were used as a covariant for ADFI analysis, while feed efficiency was used as covariant for feed efficiency analysis.

a, b, c. Means with a different superscript differ. P < 0.01.

d, e, f. Means with a different superscript differ. P < 0.05

Table 6. Contrast result of BW and ADG (covariance included).

	No Zinc		Zinc		Linear Pep * Zinc	Quad. Pep * Zinc
	Linear Pep	Quad. Pep	Linear Pep	Quad. Pep		
ADG, kg/d						
d 11-21	0.1162	0.1969	<.0001	0.4313	0.0182	0.1473
d 21-40	0.5351	0.0211	0.8307	0.4554	0.5512	0.0335
d 11-40	0.8813	0.0053	0.0638	0.5493	0.1948	0.0174
BW, kg						
d 11	0.6229	0.7200	0.1167	0.1322	0.3998	0.1856
d 21	0.2090	0.1514	<.0001	0.2441	0.0131	0.0711
d 40	0.9527	0.0050	0.0721	0.5078	0.1880	0.0150

Table 7. Contrast result of performance (covariance included)

	No Zinc		Zinc		Linear Pep * Zinc	Quad. Pep * Zinc
	Linear Pep	Quad. Pep	Linear Pep	Quad. Pep		
ADFI, kg/d						
d 11-21	0.4539	0.9839	0.2000	0.1497	0.6407	0.3145
d 21-40	0.2725	0.0938	0.3904	0.9703	0.1808	0.2307
d 11-40	0.4271	0.1373	0.2817	0.6973	0.1927	0.1926
G:F						
d 11-21	0.0614	0.1869	0.0004	0.8139	0.1241	0.4471
d 21-40	0.3511	0.9829	0.4884	0.3583	0.2448	0.5214
d 11-40	0.1294	0.6706	0.4207	0.4444	0.6356	0.4015

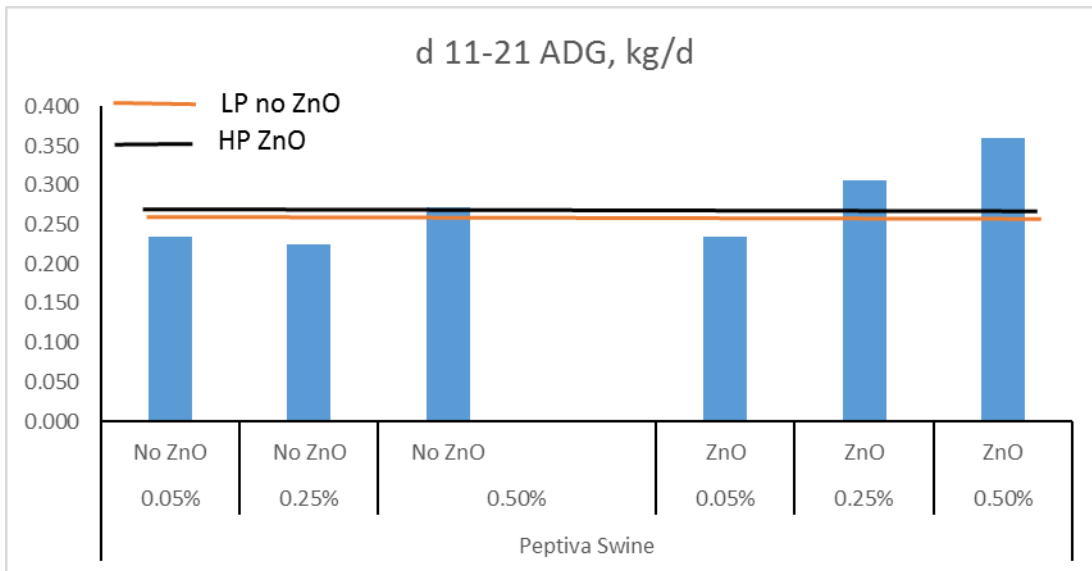


Figure 1. Effect of diet complexity and level of Peptiva Swine, with or without ZnO, on ADG (d 11-21).
 With ZnO, Linear Peptiva effect, $P < 0.001$.
 Peptiva x ZnO interaction, Linear effect, $P < 0.05$.

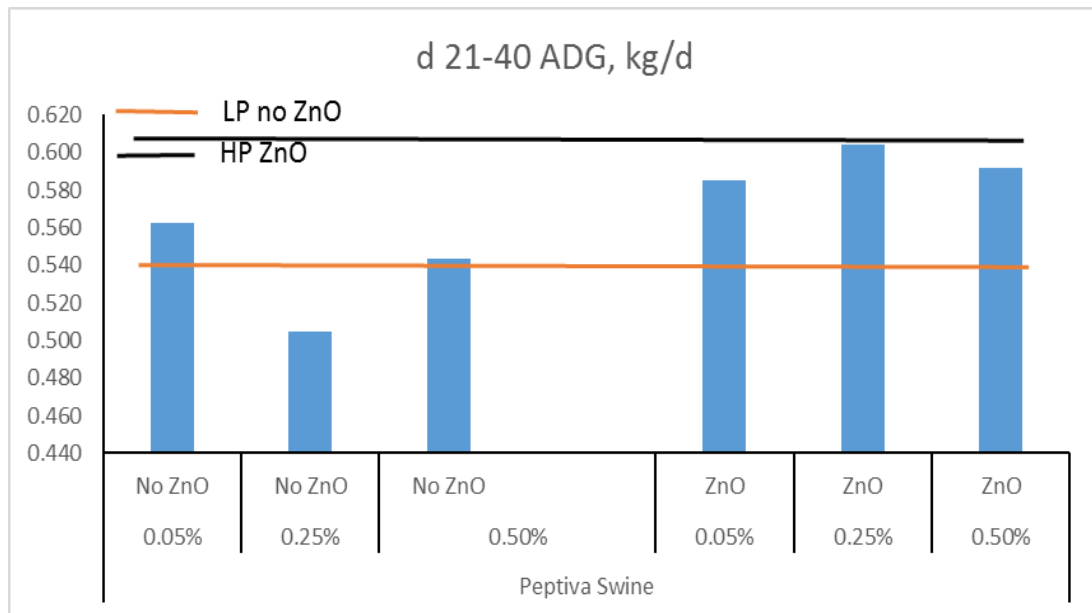


Figure 2. Effect diet complexity and level of Peptiva Swine, with or without ZnO, on ADG (d 21-40).
 Without ZnO, Quadratic effect, $P < 0.05$
 With ZnO, Linear effect, $P < 0.10$.
 Peptiva X ZnO interaction, Quadratic effect, $P < 0.05$

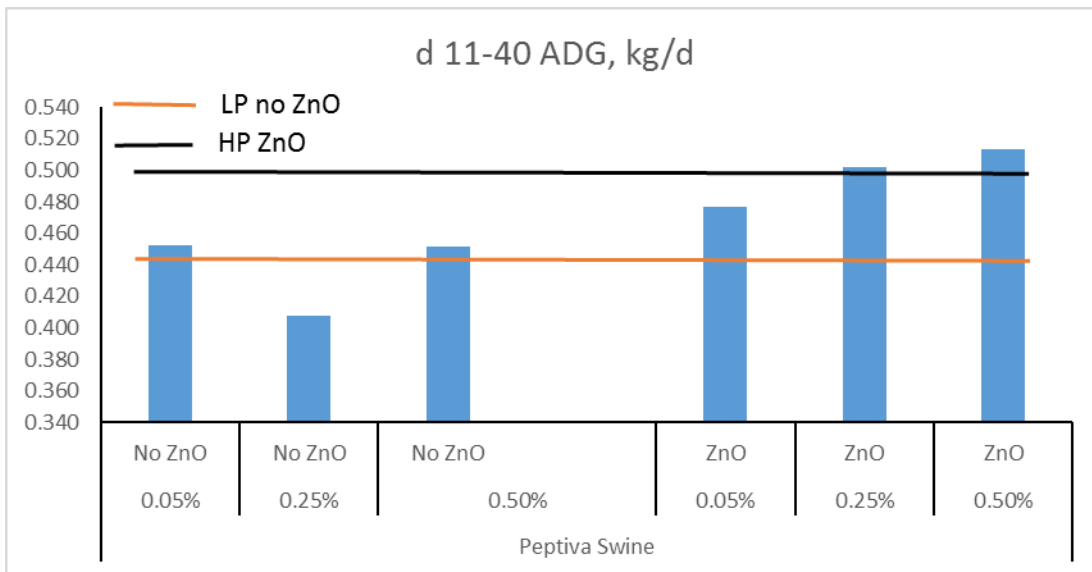


Figure 3. . Effect diet complexity and level of Peptiva Swine, with or without ZnO, on ADG (d 11-40).
 High protein diet effect, $P < 0.01$.
 Without ZnO, Quadratic effect, $P < 0.15$.
 With ZnO, Linear effect, $P < 0.10$.
 Peptiva X ZnO interaction, Quadratic effect, $P < 0.05$)

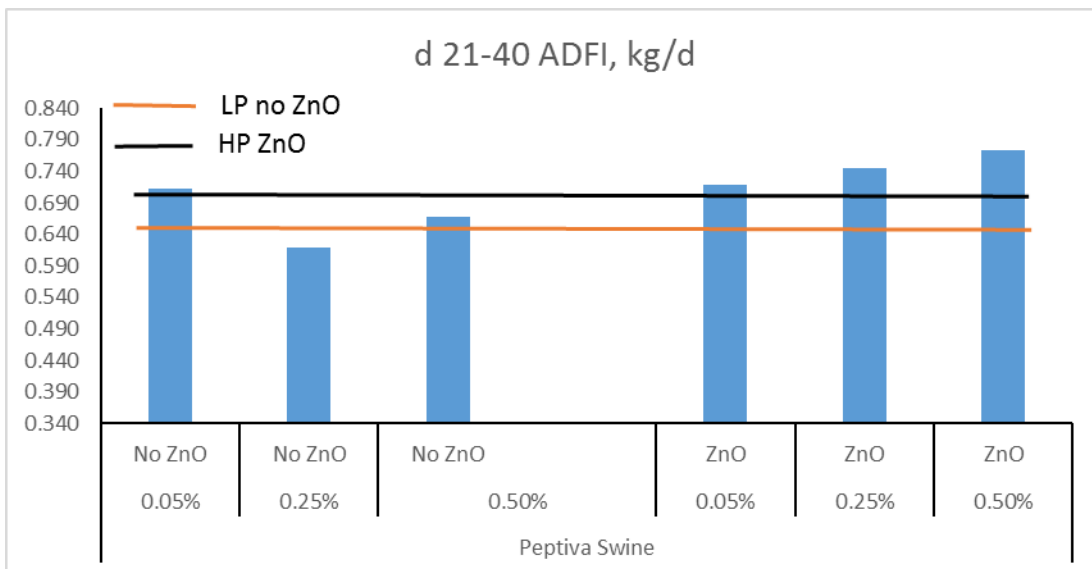


Figure 4. Effect of diet complexity and level of Peptiva Swine, with or without ZnO, on ADFI (d 21-40).
 Without ZnO, Quadratic effect, $P < 0.10$.

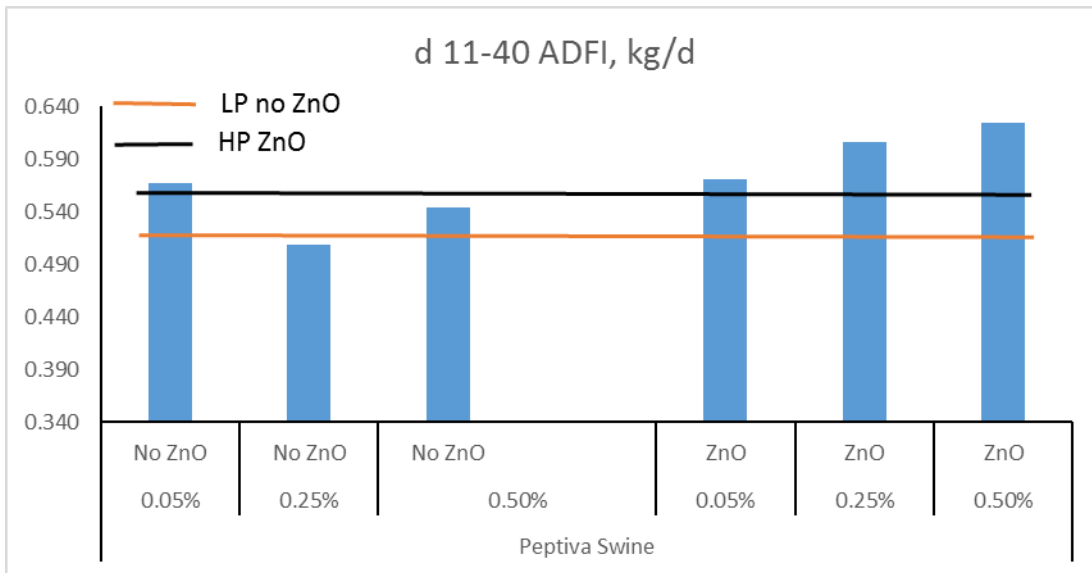


Figure 5. . Effect diet complexity and level of Peptiva Swine, with or without ZnO, on ADFI (d 11-40).

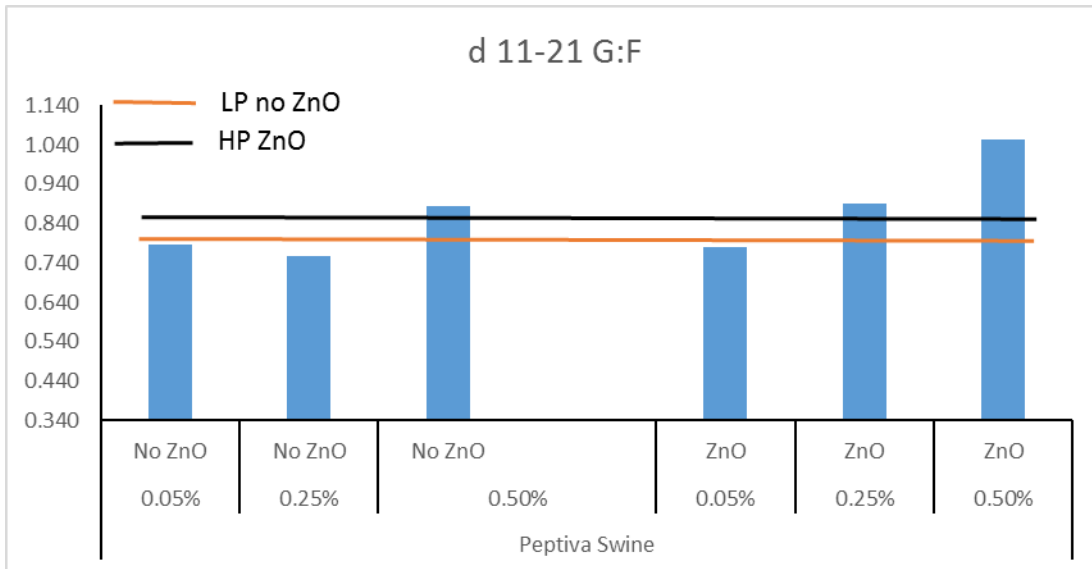


Figure 6. . Effect diet complexity and level of Peptiva Swine, with or without ZnO, on G:F (d 11-21).
 Without ZnO, Linear effect, $P < 0.10$.
 With ZnO, Linear effect, $P < 0.001$.

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