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G S. Grinstead

J Sawyer

K Maxwell

See next page for additional authors

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Influence of *Spirulina platensis* on growth performance of weanling pigs

Authors

G S. Grinstead, J Sawyer, K Maxwell, R Stott, A Moser, Michael D. Tokach, Robert D. Goodband, and Jim L. Nelssen

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INFLUENCE OF *SPIRULINA PLATENSIS* ON GROWTH PERFORMANCE OF WEANLING PIGS¹

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G. S. Grinstead, M. D. Tokach², R. D. Goodband,
J. L. Nelssen, J. Sawyer, K. Maxwell,
R. Stott, and A. Moser

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Summary

We conducted three experiments to evaluate the influence of an algal feed additive, *Spirulina platensis*, on weanling pig performance. Two experiments conducted under commercial production conditions indicated a response to *Spirulina platensis* in meal-based diets but not pelleted diets. Another experiment concluded that *Spirulina platensis* tended to improve F/G early in the trial but not for cumulative performance. Results of these three experiments suggest that *Spirulina platensis* added at low inclusions to the diet may enhance performance. However, the results lacked consistency across experiments and warrant further investigation.

(Key Words: Starter Pigs, Algae, Performance.)

Introduction

Spirulina platensis is algal derived feed additive that was first introduced to the human food market as a dietary supplement. It is organically grown in specialized ponds so it is free of contaminants. Research has indicated that *Spirulina platensis* can enhance the immune system. Growing poults fed *Spirulina platensis* had increased weight gain. These poults also had increased spleen and thymus weights. Only one experiment

with *Spirulina* has been conducted with swine. That trial evaluated *Spirulina maxima* as a replacement for dried skim milk. Results indicated that pigs fed high levels of *Spirulina maxima* (14% of the diet) had similar growth performance as those fed dried skim milk. Because of polymorphism, there is confusion regarding naming of *Spirulina*. *Maxima* and *platensis* are considered synonyms for the same plant. *Spirulina platensis* should have a pH of 10 to 11 and contain at least .9% gamma linoleic acid.

Because of the lack of data on the influence of *Spirulina platensis* in diets for pigs, we conducted these experiments to evaluate the effects of low concentrations of *Spirulina platensis* in the diet on weanling pig performance.

Procedures

Experiment 1. A total of 203 pigs (initially 8.1 lb and 11 to 12 d of age) was used in a 28-d growth trial. Pigs were blocked by weight and allotted randomly to one of four dietary treatments. There were eight or nine pigs/pen and six pens/treatment. Pigs were fed a control diet or diets containing .2, .5 or 2% *Spirulina platensis* replacing soybean meal on an equal lysine basis. The amino acid profile of *Spirulina platensis* appears to be relatively similar to that of soybean meal (Table 1). The trial was divided into three

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²Northeast Area Extension Office, Manhattan, KS.

phases to approximate a nutritional program similar to that used in commercial production: an SEW phase from d 0 to 7, a Transition phase from d 7 to 14, and Phase II from d 14 to 28. Pigs remained on their respective dietary treatments in all three phases.

Table 1. Composition of *Spirulina platensis* and Soybean Meal ^a

Item, %	<i>Spirulina platensis</i> ^b	Soybean Meal ^c
Protein	62.0	47.5
Fat	5.5	3.0
Amino Acids		
Arginine	4.30	3.48
Cystine	.60	.74
Histidine	1.00	1.28
Isoleucine	3.50	2.16
Leucine	5.40	3.66
Lysine	2.90	3.02
Methionine	1.40	.67
Phenylalanine	2.80	2.39
Threonine	3.20	1.85
Tryptophan	.90	.65
Tyrosine	3.00	1.82
Valine	4.00	2.27

^aValues shown on an as-fed basis.

^bAmino acid levels provided by Earthrise.

^cAmino acid levels were adapted from NRC (1998).

The SEW basal diet was a pelleted corn-soybean meal diet containing 6.7% spray-dried animal plasma, 1.65% spray-dried blood meal, 6% select menhaden fish meal, 25% dried whey, and 5% lactose. This diet was formulated to contain 1.6% lysine, .9% Ca, and .8% P (Table 2). The pelleted transition diet contained 2.5% spray-dried animal plasma, 2.5% spray-dried blood meal, 2.5% select menhaden fish meal, and 20% dried whey and was formulated to contain 1.5% lysine, .9% Ca, and .8% P (Table 2). The Phase II basal diet contained 2.5% spray-dried blood meal and 10% dried whey and was formulated to contain 1.3% lysine, .9% Ca, and .8% P (Table 2). The Phase II diet was the only diet fed in a meal form. *Spirulina platensis* replaced soybean meal (.2%, .5%, and 2%) in the control diet to

provide the additional experimental treatments.

Pigs were housed in an environmentally controlled nursery in 5 × 5 ft pens on a commercial farm in N.E. Kansas. All pens contained one self-feeder and two nipple waters to provide ad libitum access to feed and water. Average daily gain, ADFI, and F/G were determined by weighing pigs and measuring feed disappearance on d 7, 14, 21, and 28 after weaning.

Experiment 2. A total of 180 weanling pigs (initially 12.4 lbs. and 18d of age) was used in a 42-d growth trial to examine the effects of duration of feeding *Spirulina platensis* on growth performance. Pigs were blocked by weight and allotted randomly to one of six dietary treatments. There were five pigs/pen and six pens/treatment. The trial was divided into four phases: the SEW phase was d 0 to 7, a Transition phase from d 7 to 14, Phase II from d 14 to 28, and Phase III from d 28 to 35 after weaning. Dietary treatment groups consisted of a control group (no *Spirulina*) fed for 6 weeks; *Spirulina* (.1%) fed for 6 weeks; and *Spirulina* (.2%) fed for 1, 2, 4, or 6 weeks. At the end of each *Spirulina* feeding regimen, pigs were switched to the control diet for the remainder of the experiment.

Pigs were fed a control diet or diets containing .1 or .2% *Spirulina platensis* replacing soybean meal on an equal lysine basis. The basal diets used for the SEW, Transition, and Phase II periods had the same compositions as those used in Exp 1. The Phase III diet was a corn-soybean meal-based diet that contained 10% dried whey. Diets fed in this experiment were in a meal form and contained no medication or zinc oxide.

Pigs were housed in an environmentally controlled nursery in 5 × 5 ft pens. All pens contained one self-feeder and one nipple waterer to provide ad libitum access to feed and water. Average daily gain, ADFI, and F/G were determined by weighing pigs and measuring feed disappearance on d 7, 14, 21, 28, 35, and 42 after weaning.

Experiment 3. A total of 192 weanling pigs (initially 8.8 lbs and 11 to 12 d of age) were used in a 28-d growth trial. This experiment was implemented to examine the effects of pig performance when diets containing *Spirulina platensis* were fed in a pellet or meal form. Pigs were blocked by weight and allotted randomly to one of four dietary treatments. There were eight pigs/pen and six pens/treatment. The basal diets, feeding regimen, and experimental procedures were the same as those used in Exp.1. Pigs also were housed in the same facilities as in Exp.1. Pigs were fed a control diet in meal and pellet form or diets containing .2% *Spirulina platensis* in a pellet or meal form. *Spirulina platensis* replaced soybean meal in the control diet on an equal lysine basis.

Results and Discussion

Experiment 1. From d 0 to 7 after weaning when pigs were fed SEW diets, increasing *Spirulina platensis* had no effect on growth performance relative to those fed the control diet (Table 3). However, increasing *Spirulina platensis* tended to numerically improve ADG, ADFI, and F/G (linear, $P<.11$, $.15$, and $.15$ respectively). The linear response was predominantly within the three levels of *Spirulina platensis*, with pigs fed .2% having the poorest ADG and those fed 2.0% having the greatest ADG. Direct comparisons of each level to the control diet revealed that none of the treatments was different.

During the Transition phase (d 7 to 14 after weaning), no differences in ADG were observed. Daily feed intake increased, then decreased and then increased again (cubic, $P<.01$) in response to increasing *Spirulina platensis*. Pigs fed .2% *Spirulina platensis* had greater ADFI than those fed the control diet during this period. Feed efficiency tended to become poorer (linear, $P<.10$) with increasing *Spirulina platensis*.

From d 14 to 28 after weaning, ADG and ADFI responded in a cubic ($P<.05$) fashion with increasing *Spirulina platensis*. Pigs fed .2% *Spirulina platensis* had the best ADG

which was greater than those fed the control diet or diet containing .5% *Spirulina platensis*. Pigs fed 2.0% *Spirulina platensis* had intermediate ADG. Daily feed intake was greatest for pigs fed either .2 or 2.0% *Spirulina platensis* compared with those fed .5%, with those fed the control diet having intermediate ADFI. Feed efficiency was not affected by *Spirulina platensis*.

For the entire experimental period (d 0 to 28 after weaning), ADG (cubic, $P<.10$) and ADFI (cubic, $P<.05$) increased, then decreased and then increased again with increasing *Spirulina platensis* (Table 3). Pigs fed either .2 or 2.0% had greater ADG and ADFI than pigs fed .5% *Spirulina platensis*, whereas those fed the control diet had intermediate performance. Feed efficiency was unaffected by *Spirulina platensis*.

The results of this experiment suggest that increasing *Spirulina platensis* had no beneficial effect of growth performance during the first 2 weeks of the study when pigs were fed pelleted diets. However, from d 14 to 28, pigs fed .2% *Spirulina platensis* had increased ADG compared with those fed the control diet. The improved growth performance happens to coincide with feeding pigs meal-based diets. We speculate that perhaps high temperatures associated with the pelleting process may have damaged or inactivated the *Spirulina platensis*. Similar heat stability problems have been observed when pigs have been fed supplemental enzymes or probiotics that have been subjected to thermal processing. Because of the improved ADG observed when pigs were fed .2% *Spirulina platensis* in a meal diet, additional research warranted to confirm this positive response. The other possible explanation for the improved performance from d 14 to 28 with no response from d 0 to 14 is the duration of feeding of the *Spirulina platensis*. If the improvement in growth performance was mediated through an enhancement in immune function, a delayed response would be expected. This possibility also warranted further exploration. Therefore, Exp. 2 was conducted to examine the length of time that diets containing *Spirulina platensis* should be fed, and Exp.3 was con-

ducted to compare the effects of pig performance when pigs were fed meal or pellet diets containing *Spirulina platensis*.

Experiment 2. From d 0 to 14 after weaning, no significant differences in ADG or F/G were observed among the treatment groups. However, pigs fed either .1% or .2% *Spirulina platensis* had numerically higher ADG than pigs fed the control diet. The 20% numerical improvement in ADG was a result of an increase in ADFI ($P < .10$) found in both the .1% and .2% *Spirulina platensis* treatment groups (Table 4).

From d 7 to 14 and d 0 to 14 after weaning, no differences in ADG or ADFI were observed. However, pigs fed .2% *Spirulina platensis* for 2 weeks had higher numerical ADG than pigs fed the control diet or pigs fed the diet containing .1% *Spirulina platensis*. Pigs fed .2% *Spirulina platensis* for only 1 week had intermediate performance. Feed efficiency was not affected from d 7 to 14. However, from d 0 to 14 after weaning, pigs fed diets containing .1% or .2% *Spirulina platensis* for 2 weeks tended to have better F/G ($P < .11$) than the control group or pigs fed .2% for only 1 week.

From d 14 to 28 or d 0 to 28 after weaning, pigs fed .2% *Spirulina platensis* for only 2 weeks had higher ADFI ($P < .01$) than any other treatment group. The increase in ADFI numerically improved ADG. Pigs fed diets containing .1% or .2% *Spirulina platensis* for the entire 28 days had better feed efficiency ($P < .02$) than any other treatment group.

For the fifth and sixth week after weaning, d 28 to 42, no differences in ADG, ADFI, or F/G were observed in pigs fed the control diet; .1% *Spirulina platensis*; or .2% *Spirulina platensis* for 1, 2, 4, or 6 weeks.

For the entire experimental period (d 0 to 42 after weaning), no differences in ADG or ADFI were found. However, pigs fed .2% *Spirulina platensis* for 2 weeks tended to have numerically higher daily feed intake. Pigs fed .1% *Spirulina platensis* had numerically the best feed efficiency ($P < .09$) of any

treatment group. However, direct comparisons of the .1% *Spirulina platensis* results revealed only a difference from the .2% *Spirulina platensis* in diets fed for either 1 or 2 weeks (Table 4).

Experiment 3. From d 0 to 7 after weaning when pigs were fed SEW diets, *Spirulina platensis* had no direct effect on ADG or F/G. An effect of *Spirulina platensis* by pellet interaction ($P < .05$) on ADFI was observed. Pigs fed the pelleted *Spirulina platensis* diet had lower feed intakes, whereas pigs fed a meal *Spirulina platensis* diet had higher feed intakes. Pigs fed pelleted diets showed a significant improvement in feed efficiency ($P < .01$) (Table 5).

During the Transition period, d 7 to 14 after weaning, feeding *Spirulina platensis* had no effect on ADG, ADFI, or F/G. From d 0 to 14 after weaning, adding *Spirulina platensis* to pelleted diets tended to decrease ADG (*Spirulina* × pellet, $P < .10$), whereas adding *Spirulina platensis* to meal diets tended to slightly improve ADG. Pigs fed *Spirulina platensis* in a pellet form had lower ADFI ($P < .06$) than any other treatment group. Pigs fed pelleted diets had better F/G ($P < .01$) than pigs fed meal-based diets.

From d 14 to 28 after weaning, ADG, ADFI, and F/G were not affected significantly by *Spirulina platensis*. However, pigs fed *Spirulina platensis* had numerically better ADG compared to the pigs fed the control diets. For the entire experimental period, d 0 to 28 after weaning, *Spirulina platensis* fed in either a meal or pellet form had no effect on ADFI or F/G. Average daily gain means tended to be greater in pigs fed *Spirulina platensis*. Pig weights also tended to be slightly higher in pigs fed *Spirulina platensis*, with the heaviest pigs from the *Spirulina platensis* meal diet. A pellet by *Spirulina platensis* interaction occurred for pig weight on d 14 ($P < .05$) and 21 ($P < .04$). Adding *Spirulina platensis* to pelleted diets decreased pig weight, whereas adding it to meal diets increased pig weight.

The improvements in pig performance from feeding *Spirulina platensis* were predominately in meal-based diets. This suggests that *Spirulina platensis* may not be heat stable under pelleting processes. The results of these three experiments suggest that

Spirulina platensis, added at low inclusions to the diet, can enhance performance. However, the improvements lack consistency across experiments. These inconsistencies warrant further investigation.

Table 2. Compositions of Basal Diets

Ingredient, %	Dietary Phases			
	SEW ^a	Transition ^b	Phase II ^c	Phase III ^d
Corn	33.57	39.88	58.18	65.06
Dried whey	25.00	20.00	10.00	10.00
Soybean meal ^e	12.47	23.24	24.56	30.54
Spray-dried animal plasma	6.70	2.50	---	---
Soybean oil	6.00	5.00	---	---
Select menhaden fish meal	6.00	2.50	---	---
Lactose	5.00	---	---	---
Spray-dried blood meal	1.75	2.50	2.50	2.50
Antibiotic ^f	1.00	1.00	1.00	---
Monocalcium phosphate	.76	1.28	1.64	1.53
Limestone	.48	.75	1.00	.97
Zinc oxide	.38	.38	.25	---
Vitamin premix	.25	.30	.25	.25
Salt	.20	.25	.25	.35
L-Lysine HCl	.15	.15	.15	.15
Trace mineral premix	.15	.15	.15	.15
DL-methionine	.15	.13	.08	.01
Total	100.00	100.00	100.00	100.00

^aDiets were formulated to contain 1.7% lysine, .48% methionine, .9% Ca, .8% P and were fed from d 0 to 7 postweaning.

^bDiets were formulated to contain 1.6% lysine, .44% methionine, .9% Ca, .8% P and were fed from d 7 to 14 postweaning.

^cDiets were formulated to contain 1.3% lysine, .36% methionine, .85% Ca, .75% P and were fed from 14 to 28 postweaning.

^dDiets were formulated to contain 1.2% lysine, .32% methionine, .75% Ca, .70% P and were fed from d 28 to 42 postweaning (Exp.2).

^e*Spirulina platensis* replaced soybean meal on an equal lysine basis.

^f Provided 50 g/ton carbodox.

Table 3. Effect of Increasing *Spirulina* Fed from d 0 to 28 after Weaning on Pig Performance (Exp.1)^a

Item	Control	<i>Spirulina</i> , %			SEM	P <		
		.2	.5	2.0		Lin.	Quad.	Cubic
Day 0 to 7								
ADG, lb	.28 ^{ab}	.25 ^a	.27 ^{ab}	.31 ^b	.02	.11	.45	.35
ADFI, lb	.32 ^{ab}	.31 ^a	.32 ^{ab}	.33 ^b	.01	.15	.79	.36
F/G	1.14 ^{ab}	1.22 ^a	1.18 ^{ab}	1.07 ^b	.06	.15	.39	.42
Day 7 to 14								
ADG, lb	.49	.52	.53	.48	.02	.44	.68	.22
ADFI, lb	.53 ^a	.58 ^b	.54 ^{ab}	.56 ^{ab}	.02	.69	.76	.10
F/G	1.07 ^a	1.10 ^{ab}	1.14 ^{ab}	1.18 ^b	.05	.10	.38	.96
Day 0 to 14								
ADG, lb	.39	.39	.37	.39	.02	.73	.51	.72
ADFI, lb	.42	.44	.43	.44	.01	.40	.87	.31
F/G	1.09	1.14	1.16	1.14	.03	.55	.25	.70
Day 14 to 21								
ADG, lb	.53	.55	.46	.53	.03	.92	.17	.25
ADFI, lb	.84 ^a	.87 ^a	.77 ^b	.89 ^a	.02	.12	.02	.07
F/G	1.59	1.59	1.66	1.65	.06	.67	.66	.75
Day 21 to 28								
ADG, lb	.74 ^c	.88 ^d	.82 ^{cd}	.83 ^{cd}	.04	.55	.25	.06
ADFI, lb	1.18 ^a	1.29 ^b	1.18 ^a	1.25 ^{ab}	.03	.52	.83	.03
F/G	1.61 ^c	1.47 ^d	1.45 ^d	1.51 ^{cd}	.04	.56	.02	.30
Day 14 to 28								
ADG, lb	.63 ^c	.71 ^d	.64 ^c	.68 ^{cd}	.02	.62	.99	.04
ADFI, lb	1.01 ^{cd}	1.08 ^c	.98 ^d	1.07 ^c	.03	.28	.24	.03
F/G	1.60	1.52	1.52	1.56	.05	.97	.26	.44
Day 0 to 28								
ADG, lb	.51 ^{ab}	.55 ^a	.51 ^b	.53 ^{ab}	.01	.60	.73	.08
ADFI, lb	.72 ^{ab}	.76 ^a	.70 ^b	.75 ^a	.02	.34	.41	.05
F/G	1.40	1.39	1.39	1.40	.02	.74	.56	.70
Avg. Pig wt, lb								
d 0	8.08	8.11	8.07	8.08	.03	.51	.16	.52
d 7	10.02 ^{ab}	9.85 ^a	9.95 ^{ab}	10.24 ^b	.16	.13	.51	.28
d 14	13.47	13.51	13.27	13.57	.29	.47	.63	.81
d 21	17.20	17.34	16.51	17.62	.33	.45	.83	.76
d 28	22.37	23.49	22.22	23.41	.51	.43	.48	.28

^aA total of 203 weanling pigs (initially 8.1 lb and 11 to 12 d of age) with 8 or 9 pigs per pen and six replications per treatment.

^{ab}Means in the same row with different letters are different (P<.10).

^{cd}Means in the same row with different letters are different (P<.05).

Table 4. Effects of Feeding Duration of *Spirulina platensis* on Weanling Pig Performance (Exp.2)¹

Item	0	.1%	.2% ²	<i>Spirulina</i> Removed on Day 3			SEM	P <
				7	14	28		
Day 0 to 7								
ADG, lb	.23	.29	.29				.028	.32
ADFI, lb	.28 ^a	.35 ^b	.34 ^b				.020	.07
F/G	1.25	1.23	1.25				.112	.99
Day 7 to 14								
ADG, lb	.62	.61	.68	.66			.032	.26
ADFI, lb	.71	.65	.74	.77			.041	.29
F/G	1.15	1.06	1.09	1.18			.039	.20
Day 0 to 14								
ADG, lb	.42	.45	.49	.46			.027	.29
ADFI, lb	.50	.50	.54	.54			.027	.40
F/G	1.17	1.11	1.11	1.19			.028	.11
Day 14 to 28								
ADG, lb	1.07	1.06	1.06	1.05	1.11		.028	.72
ADFI, lb	1.60 ^{cd}	1.51 ^c	1.57 ^{cd}	1.64 ^d	1.77 ^e		.042	.004
F/G	1.52 ^{ce}	1.41 ^d	1.47 ^{cd}	1.56 ^c	1.60 ^e		.037	.02
Day 0 to 28								
ADG, lb	.74	.76	.77	.76	.81		.024	.46
ADFI, lb	1.05 ^{cd}	1.01 ^c	1.04 ^{cd}	1.09 ^d	1.18 ^e		.032	.01
F/G	1.42 ^c	1.32 ^d	1.35 ^d	1.44 ^c	1.46 ^c		.026	.004
Day 28 to 42								
ADG, lb	1.55	1.55	1.49	1.55	1.56	1.51	.049	.89
ADFI, lb	2.52	2.51	2.45	2.56	2.55	2.53	.061	.80
F/G	1.63	1.63	1.65	1.66	1.64	1.68	.025	.68
Day 0 to 42								
ADG, lb	1.01	1.02	1.00	1.02	1.06	1.01	.029	.81
ADFI, lb	1.54	1.51	1.51	1.58	1.64	1.52	.037	.14
F/G	1.52 ^{cd}	1.48 ^c	1.50 ^c	1.55 ^d	1.55 ^d	1.50 ^c	.020	.09
Avg. pig wt, lb								
d 0	12.46	12.43	12.42				.025	.47
d 7	14.10	14.47	14.44				.055	.40
d 14	18.40	18.78	19.25	18.96			.380	.36
d 21	23.55	23.98	24.46	24.21	25.72		.585	.17
d 28	33.32	33.68	34.56	33.72	35.04		.928	.69
d 35	43.29	44.18	45.68	43.78	45.67	45.61	1.27	.61
d 42	55.00	55.33	56.17	55.37	56.83	56.34	1.42	.93

¹A total of 180 weanling pigs (initially 12.4 lb and 18 d of age) with five pigs per pen and six replications per treatment.

²Represents mean of all pens remaining on .2% *Spirulina* for each respective weight period.

³Represents mean of all pens previously fed .2% *Spirulina* then switched to the control diet.

^{a,b}Means in the same row with different letters are different (P<.05).

^{c,d,e}Means in the same row with different letters are different (P<.10).

Table 5. Effects of Feeding *Spirulina platensis* in a Meal or Pelleted Diet on Weanling Pig Performance (Exp.3)¹

Item	Meal		Pelleted		SEM	P <		
	Control	<i>Spirulina</i>	Control	<i>Spirulina</i>		Pellet	<i>Spirulina</i>	Spir × Pell ²
Day 0 to 7								
ADG, lb	.24	.25	.31	.28	.023	.07	.84	.29
ADFI, lb	.37 ^a	.39 ^b	.35 ^a	.32 ^c	.012	.009	.70	.03
F/G	1.55	1.55	1.16	1.20	.096	.002	.84	.82
Day 7 to 14								
ADG, lb	.56	.57	.55	.55	.024	.36	.74	.68
ADFI, lb	.76	.76	.70	.65	.029	.002	.36	.41
F/G	1.36	1.35	1.26	1.19	.063	.02	.42	.51
Day 0 to 14								
ADG, lb	.39 ^d	.41 ^{de}	.43 ^c	.41 ^{de}	.012	.15	.89	.10
ADFI, lb	.56 ^a	.57 ^a	.52 ^b	.49 ^c	.014	.001	.50	.06
F/G	1.41	1.40	1.21	1.18	.043	.001	.61	.80
Day 14 to 28								
ADG, lb	.75	.77	.69	.73	.023	.05	.19	.67
ADFI, lb	1.08	1.10	1.01	1.04	.025	.02	.38	.98
F/G	1.45	1.44	1.48	1.42	.046	.97	.46	.69
Day 0 to 28								
ADG, lb	.57	.59	.56	.57	.012	.16	.18	.78
ADFI, lb	.82	.84	.76	.76	.015	.001	.60	.49
F/G	1.44	1.43	1.37	1.33	.036	.05	.50	.71
Avg. pig wt, lb								
d 0	8.82	8.83	8.81	8.84	.008	.99	.89	.33
d 7	10.44	10.62	10.98	10.78	.178	.07	.95	.31
d 14	14.33 ^a	14.63 ^a	15.11 ^b	14.61 ^a	.192	.07	.60	.05
d 21	17.82 ^a	18.89 ^b	19.05 ^b	18.50 ^{ab}	.359	.26	.49	.04
d 28	24.76	25.36	24.73	24.79	.411	.48	.44	.53

¹A total of 180 weanling pigs (initially 8.8 lbs and 11 to 12 d of age) with eight pigs per pen and six replications per treatment.

²Interaction of *Spirulina* and pellets.

^{abc}Means in the same row are different (P<.10).

^{de}Means in the same row are different (P<.05).