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Effects of Diet and Water Monoglyceride and Acid Supplementation on Nursery Pig Performance

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Ty H. Kim, Jason C. Woodworth, Mike D. Tokach, Joel M. DeRouchey, Robert D. Goodband, Katelyn N. Gaffield, Jordan T. Gebhardt,[1] Ying Chen,[2] and Mark Whitney[2]

Summary

A total of 2,520 pigs (PIC 337 × 1050, initially 11.5 ± 0.22 lb) were used in a 42-d study to determine the effects of dietary and water-based monoglyceride and acid supplementation on growth performance of nursery pigs. Pigs were weaned at approximately 20 d of age and randomly allotted to pens. Pens of pigs were blocked by initial BW and allotted to one of six treatments in a randomized complete block design with 30 pigs per pen and 14 pens per treatment across two rooms. Treatment diets were formulated in three phases and fed based on a feed budget. The six treatments were arranged in a 2 × 3 factorial with main effects of acidifier supplementation in water (Control or Protaq Bond 50) and feed (Control, Acitra G20C, or Acitra G20C and Entero-Nova 410C). Protaq Bond 50 is a blend of organic acids and monoglycerides applied to the water source through the water medicator included at a rate to reach a target water pH of 4.0. Acitra G20C, a blend of organic acids, was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3. Entero-Nova 410C, a blend of monoglycerides, was included at 0.3% in phases 1 and 2, and 0.1% in phase 3. Pigs were weighed and feed disappearance was measured every 7 d to determine ADG, ADFI, and F/G. From d 0 to 7, pigs provided water with Protaq Bond 50 had increased ($P < 0.001$) ADG and improved ($P < 0.001$) F/G compared to pigs provided control water. Over the entire 42-d period, there were no differences ($P > 0.10$) in ADG or ADFI for water or feed treatments. There was a tendency ($P = 0.098$) for a main effect of diet on F/G, where pigs fed Acitra G20C had the lowest numerical F/G and pigs fed the control diet had the highest numerical F/G. For mortality, there was a tendency ($P = 0.055$) for a water × diet interaction, where diet did not affect mortality in pigs provided the control water; however, when provided Protaq Bond 50 in the water, pigs fed Acitra G20C had numerically lower mortality, while pigs fed Acitra G20C and Entero-Nova 410C had numerically higher mortality compared to pigs fed the control. There were no differences ($P > 0.10$) in removals or total removals and mortality. In summary, pigs receiving Protaq water had improved ADG and F/G compared to pigs on the control water in the first 7 d post-weaning. Pigs fed a blend of organic acids had numerically improved F/G compared to pigs fed the control, but there was no further

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benefit when both organic acids and monoglycerides were included in the diet. The addition of monoglycerides and other acidifiers in feed or water had no effect on overall ADG, ADFI, removals, or total removals and mortality.

Introduction

Newly weaned pigs often exhibit poor performance and diarrhea in the early nursery period due to their inability to produce enough hydrochloric acid to lower gastric pH, which can result in poor nutrient utilization and proliferation of bacteria.[3] The addition of organic acids and/or monoglycerides in nursery diets is a strategy to lower intestinal pH to improve gut health and performance. Organic acids act by reducing the pH of the digestive tract, which can improve growth performance of pigs by improving gut morphology, increasing nutrient digestibility, promoting beneficial bacteria, and inhibiting harmful bacteria.[4] Monoglycerides of fatty acids can also positively alter the gut microbial population and lead to improved gut health.[5] A water-to-feed intake ratio of 2:1 to 3:1 is typical in nursery pigs.[6] As a result, supplementing acidifiers, such as monoglycerides, via water delivery could increase acid intake and lower the gastric pH further. However, there is limited research on the combination of these additives delivered via the diet and water. Therefore, the objective of this study was to evaluate the effects of diet and water monoglyceride and acid supplementation on nursery pig performance and fecal dry matter (DM).

Materials and Methods

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The experiment was conducted at a commercial research site in southwest Minnesota. Each pen was equipped with a six-hole, dry self-feeder and pan waterer to provide *ad libitum* access to feed and water. Phase 1 diets were manufactured at Hubbard Feeds (Mankato, MN) and were in pellet form. Phase 2 and 3 diets were manufactured at the Hord Farms West Feed Mill (Pipestone, MN) and were in mash form. Daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) that recorded feed deliveries for individual pens. Neomycin was supplied to all pigs at placement via water lines. Penicillin G potassium was supplied to all pigs, via water lines, on approximately d 10 post-placement. Pigs were vaccinated via intramuscular injection for circovirus, mycoplasma, PRRSv, and ileitis on approximately d 17 post-placement.

A total of 2,520 pigs (PIC 337 × 1050, initially 11.5 ± 0.22 lb) were used in a 42-d study with 30 pigs per pen and 14 replications per treatment across two rooms. Pigs were weaned at approximately 20 d of age and randomly allotted to pens. Pens of pigs were blocked by initial BW and allotted to one of six treatments in a randomized complete block design. Treatment diets were formulated in three phases and fed based on feed budgets of 4, 12, and ~40 lb/pig, respectively. The six treatments were

[3] Tsiloyiannis, V. K., S. C. Kyriakis, J. Vlemmas. 2001. The effect of organic acids on the control of porcine post-weaning diarrhoea. Res. Vet. Sci. 70:287-293. doi: 10.1053/rvsc.2001.0476.
[4] Suiryanrayna, M. V. A. N., J. V. Ramana. 2015. A review of the effects of dietary organic acids fed to swine. J. Anim. Sci. Biotechnol. 6:45. doi: 10.1186/s40104-015-0042-z.
[5] Jackman J. A., R. D. Boyd, C. C. Elrod. 2020. Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. J Anim Sci Biotechnol. 11:44. doi: 10.1186/s40104-020-00446-1.
[6] Shaw, M. I., A. D. Beaulieu, and J. F. Patience. 2006. Effect of diet composition on water consumption in growing pigs. J. Anim. Sci. 84:3123-3132. doi:10.2527/jas.2005 690.

arranged in a 2 × 3 factorial design with main effects of acid supplementation in water (Control or Protaq Bond 50) and feed (Control, Acitra G20C, or Acitra G20C and Entero-Nova 410C). Protaq Bond 50 is a blend of organic acids and monoglycerides applied to the water source through the water medicator included at a rate to reach a target water pH of 4.0. For the pigs on the Protaq Bond 50 water treatment, Protaq Bond 50 was applied to the water throughout all three phases of the study. Water pH was measured at drinkers at the end of water lines, using a pH electrode, at d 10, 24, and 42 for both water treatments (Table 1). Acitra G20C, a blend of organic acids, was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3. Entero-Nova 410C, a blend of monoglycerides, was included at 0.3% in phases 1 and 2, and 0.1% in phase 3. Pigs were weighed and feed disappearance was measured every 7 d to determine ADG, ADFI, and F/G.

Fecal samples were collected at the end of each dietary phase on approximately d 10, 24, and 42 to determine fecal dry matter (DM) percentage from the same three medium-weight pigs from each pen on each collection day. After collection, fecal samples were dried at 55°C (131°F) in a forced air oven and the ratio of dried-to-wet fecal weight determined the fecal DM percentage.

Data analysis

Growth data were analyzed as a randomized complete block design with pen serving as the experimental unit and block and room serving as random intercepts. The main effects of water and diet acid supplementation, as well as their interactions, were tested. Growth data were analyzed using the lmer package of R (version 4.2.2 (2022-10-31), R Core Team, Vienna, Austria). For fecal DM, data were analyzed as a randomized complete block design with pen as the experimental unit and day, diet, water, and the associated interaction included as fixed effects accounting for repeated measures over time. Pen was included in the model as a random intercept to account for multiple observations for each pen on each day. Fecal DM data were analyzed using the GLIMMIX procedures of SAS (version 9.4; Cary, NC). Differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

There were no diet × water acidification interactions ($P > 0.10$; Table 3) observed for growth performance measures throughout the study. From d 0 to 7, there was a main effect of water acid supplementation resulting in increased ADG and d 7 BW and improved F/G ($P < 0.001$; Table 5) in pigs provided Protaq Bond 50 in the water compared to those that were provided control water. There was a tendency ($P = 0.056$; Table 4) for a main effect of diet organic acid and monoglyceride supplementation on ADFI with pigs fed the control, and Acitra G20C+Entero-Nova 410C diets having higher ADFI than pigs fed the diet containing only Acitra G20C.

From d 7 to 21, there was a main effect of water organic acid and monoglyceride supplementation, resulting in increased ($P < 0.05$) ADG and ADFI when pigs were offered water without Protaq Bond 50. There was also a main effect of diet organic acid and monoglyceride supplementation on F/G ($P = 0.042$), with pigs fed the diet with Acitra G20C+Entero-Nova 410C being the most efficient.

From d 21 to 42 (phase 3), there was a main effect of diet organic acid and monoglyceride supplementation, resulting in increased ADG and improved F/G ($P < 0.05$)

in pigs previously fed the Acitra G20C diet compared to pigs fed the control and Acitra G20C+Entero-Nova 410C diets.

From d 0 to 42, there were no differences ($P > 0.10$) observed for ADG or ADFI for water or dietary treatments. There was a tendency ($P = 0.098$) for a main effect of diet on F/G, where pigs fed Acitra G20C in phases 1 and 2 had the numerically lowest F/G, while pigs fed the control had the highest.

For mortality, there was a tendency ($P = 0.055$) for a water × diet interaction, where diet acidification did not affect mortality when pigs were offered control water; however, when offered Protaq Bond 50 in the water, pigs fed Acitra G20C had numerically lower mortality, while pigs fed Acitra G20C+Entero-Nova 410C had numerically higher mortality compared to pigs fed the control. There were no treatment differences ($P > 0.10$) in removals or total removals and mortality.

For fecal DM, there was a tendency for a diet × water acidification interaction ($P = 0.095$; Table 6) on d 24, where pigs fed Acitra G20C and Acitra G20C+Entero-Nova 410C diets had numerically lower fecal DM compared to control-fed pigs when provided control water; however, when pigs were provided Protaq Bond 50 supplemented water, pigs fed Acitra G20C and Acitra G20C+Entero-Nova 410C diets had numerically higher fecal DM compared to control-fed pigs. There was a diet × water acidification interaction ($P = 0.015$) on d 42, where there were no differences in fecal DM when pigs were provided control water; however, when pigs were provided Protaq Bond 50 supplemented water, pigs fed the control diet had numerically lower fecal DM compared to pigs fed Acitra G20C and Acitra G20C+Entero-Nova 410C diets. There was a tendency ($P = 0.076$) for a main effect of water treatment on d 42 fecal DM, with pigs provided control water having numerically higher fecal DM than pigs provided Protaq Bond 50 in water. There was a main effect of diet ($P = 0.005$) on d 42 fecal DM, with pigs fed the Acitra G20C diet having higher fecal DM than pigs fed the control diet.

In summary, pigs provided Protaq Bond 50 in the water had improved ADG and F/G compared to pigs on the control water for the first 7 d post-weaning. Pigs fed a blend of organic acids had numerically improved F/G compared to pigs fed the control diet, but there was no further benefit when both organic acids and monoglycerides were included in the diet. The addition of monoglycerides and other acidifiers in feed or water had no effect on overall ADG, ADFI, removals, or total removals and mortality. Pigs fed organic acids or organic acids and monoglycerides had numerically higher fecal DM on d 42 compared to pigs fed the control when supplemented with organic acids and monoglycerides in the water. Additionally, pigs fed organic acids had higher fecal DM compared to pigs fed the control on d 42.

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Appreciation is expressed to Eastman Chemical Company (Kingsport, TN) and Hord Farms West (Pipestone, MN) who provided partial financial and technical support for this project.

Table 1. Water pH[1]

Item	Control	Protaq[2]
d 10	7.08	4.05
d 24	7.48	4.01
d 42	7.28	4.15

[1]Water pH was measured at drinkers at the end of water lines, using a pH electrode, at d 10, 24, and 42 for both water treatments. Values represent the average of four measurements at each timepoint.
[2]Protaq Bond 50 (liquid blend of organic acids and monoglycerides) was included in the water, via a water medicator, at a rate to reach a target pH of 4.0.

Table 2. Composition of experimental diets (as-fed basis)[1]

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	40.95	58.68	55.52
Soybean meal	16.85	27.74	30.03
Whey powder	25.00	---	---
Dried distillers grains with solubles	5.00	7.50	10.00
Microbially enhanced soybean meal[2]	5.00	---	---
Corn oil	2.00	---	---
Limestone	0.70	0.65	0.62
Monocalcium phosphate	1.10	1.35	0.70
Salt	0.15	0.45	0.29
L-Lys-HCl	0.48	---	---
Liquid Lys (55%)	---	0.85	0.64
DL-Met	0.26	0.25	0.18
L-Thr	0.19	---	---
Thr-Pro[3]	---	0.32	0.21
L-Trp	0.03	0.03	---
L-Val	0.12	0.15	0.05
Vitamin premix	0.05	---	---
Trace mineral premix	0.08	---	---
Vitamin-trace mineral premix	---	0.13	0.13
Choline chloride	0.04	---	---
Selenium premix	0.05	---	---
Phytase[4]	0.05	0.08	0.08
Zinc oxide	0.40	0.27	---
Sodium metabisulfite	0.50	---	---
Feed Aid[5]	---	0.55	0.55
Mecadox[6]	1.00	1.00	1.00
Total	100	100	100

continued

Table 2. Composition of experimental diets (as-fed basis)[1]

Ingredient, %	Phase 1	Phase 2	Phase 3
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.35	1.35	1.30
Ile:Lys	57	54	60
Leu:Lys	118	117	131
Met:Lys	39	39	37
Met and Cys:Lys	60	60	60
Thr:Lys	65	65	65
Trp:Lys	20	20	21
Val:Lys	69	70	70
His:Lys	33	36	40
NE, kcal/lb	1,152	1,072	1,074
CP, %	20.3	21.4	22.6
Ca, %	0.71	0.71	0.59
STTD P, %	0.62	0.56	0.45
Ca:P	1.04	1.04	1.04
Zn, ppm	3,000	2,000	110
Analyzed values, %[7]			
DM	87.7	87.0	86.9
CP	19.9	21.2	22.9

[1]Phase 1 and 2 diets were provided with a feed budget of 4 and 12 lb/pig, respectively. For the Acitra G20C diet, Acitra G20C (a blend of organic acids; Eastman Chemical Company, Kingsport, TN) was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3 at the expense of corn. For the Acitra G20C+Entero-Nova 410C diet, Acitra G20C was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3, and Entero-Nova 410C (a blend of monoglycerides; Eastman Chemical Company, Kingsport, TN) was included at 0.3% in phases 1 and 2, and 0.1% in phase 3 at the expense of corn.
[2]ME-PRO, Prairie Aquatech, Brookings, SD.
[3]CJ America Bio, Downers Grove, IL.
[4]Phase 1: Quantum Blue 5G (ABVista, Plantation, FL), which provided an estimated release of 0.12% STTD P with 1,135 FTU/lb. Phase 2 and 3: Optiphos Plus 2500 G (Huvepharma Inc.; Peachtree City, Georgia), which provided an estimated release of 0.13% STTD P with 908 FTU/lb.
[5]NutriQuest, Mason City, IA.
[6]Mecadox (Phibro Animal Health Corp., Teaneck, NJ) provided 55 ppm of carbadox in the diet.
[7]Complete diet samples were taken during bagging of experimental diets from every fourth bag and pooled into one homogenized sample per dietary treatment. Samples were stored at -4°F until they were analyzed for DM and CP in the Kansas State University Swine Laboratory.

Table 3. Effects of diet and water monoglyceride and acid supplementation on growth performance of nursery pigs[1]

Item	Water:	Control			Protaq[2]				*P* =		
	Diet:	Control	Acitra[3]	Acitra + EN[4]	Control	Acitra	Acitra + EN	SEM	Diet × water	Water	Diet
BW, lb											
d 0		11.5	11.5	11.5	11.5	11.5	11.5	0.22	0.992	0.943	0.983
d 7		12.7	12.7	12.6	13.0	13.0	12.9	0.24	0.998	0.001	0.556
d 21		24.6	24.5	24.6	24.6	24.4	24.3	0.48	0.519	0.478	0.719
d 42		52.3	52.2	51.8	52.1	52.5	51.6	0.88	0.777	0.924	0.257
d 0 to 7											
ADG, lb		0.16	0.16	0.15	0.20	0.20	0.19	0.013	0.971	< 0.001	0.272
ADFI, lb		0.31	0.30	0.31	0.31	0.30	0.30	0.009	0.651	0.670	0.056
F/G		2.03	2.01	2.77	1.69	1.54	1.64	0.300	0.866	< 0.001	0.153
d 7 to 21											
ADG, lb		0.85	0.85	0.86	0.84	0.83	0.82	0.019	0.623	0.020	0.769
ADFI, lb		0.98	0.99	0.96	0.96	0.95	0.94	0.021	0.739	0.043	0.290
F/G		1.15	1.17	1.12	1.15	1.15	1.14	0.014	0.259	0.894	0.042
d 21 to 42											
ADG, lb		1.32	1.32	1.29	1.30	1.33	1.30	0.027	0.573	0.892	0.038
ADFI, lb		1.95	1.92	1.93	1.95	1.95	1.91	0.057	0.451	0.717	0.416
F/G		1.48	1.45	1.49	1.49	1.47	1.47	0.017	0.122	0.641	0.043
d 0 to 42											
ADG, lb		0.95	0.96	0.94	0.95	0.96	0.94	0.020	0.917	0.689	0.131
ADFI, lb		1.33	1.32	1.31	1.33	1.33	1.30	0.034	0.821	0.830	0.249
F/G		1.40	1.38	1.40	1.40	1.38	1.38	0.011	0.398	0.277	0.098
Removals, %		6.0	4.1	6.9	4.8	4.3	2.6	1.20	0.116	0.450	0.182
Mortality, %		1.4	1.6	1.1	1.4	0.7	2.8	0.80	0.055	1.000	0.830
Total removals and mortality, %		7.4	5.7	8.1	6.2	5.0	5.5	1.40	0.761	0.531	0.437

[1]A total of 2,520 pigs (PIC 337 × 1050; initially 11.5 ± 0.22 lb) were used in a 42-d study with 30 pigs per pen and 14 replications per treatment across two rooms.
[2]Protaq Bond 50 (liquid blend of organic acids and monoglycerides) was included in the water, via a water medicator, at a rate to reach a target pH of 4.0.
[3]Acitra G20C (blend of organic acids) was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3.
[4]Acitra G20C was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3. Entero-Nova 410C (blend of monoglycerides) was included at 0.3% in phases 1 and 2, and 0.1% in phase 3.

Table 4. Main effect of diet organic acid and monoglyceride supplementation on growth performance of nursery pigs[1]

Item	Control	Acitra[2]	Acitra + EN[3]	SEM	*P* =
BW, lb					
d 0	11.5	11.5	11.5	0.21	0.983
d 7	12.8	12.8	12.7	0.23	0.556
d 21	24.6	24.5	24.5	0.46	0.719
d 42	52.2	52.4	51.7	0.83	0.257
d 0 to 7					
ADG, lb	0.18	0.18	0.17	0.011	0.272
ADFI, lb	0.31	0.30	0.31	0.007	0.056
F/G	1.86	1.78	2.20	0.248	0.153
d 7 to 21					
ADG, lb	0.85	0.84	0.84	0.017	0.769
ADFI, lb	0.97	0.97	0.95	0.018	0.290
F/G	1.15	1.16	1.13	0.010	0.042
d 21 to 42					
ADG, lb	1.31[ab]	1.32[a]	1.29[b]	0.025	0.038
ADFI, lb	1.95	1.94	1.92	0.055	0.416
F/G	1.49	1.46	1.48	0.015	0.043
d 0 to 42					
ADG, lb	0.95	0.96	0.94	0.018	0.131
ADFI, lb	1.33	1.32	1.31	0.033	0.249
F/G	1.40	1.38	1.39	0.009	0.098
Removals, %	5.4	4.2	4.8	0.87	0.182
Mortality, %	1.4	1.1	2.0	0.71	0.830
Total removals and mortality, %	6.8	5.4	6.8	0.97	0.437

[a,b]Means within row with different superscripts differ ($P < 0.05$).
[1]A total of 2,520 pigs (PIC 337 × 1050; initially 11.5 ± 0.21 lb) were used in a 42-d study.
[2]Acitra G20C (blend of organic acids) was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3.
[3]Acitra G20C was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3. Entero-Nova 410C (blend of monoglycerides) was included at 0.3% in phases 1 and 2, and 0.1% in phase 3.

Table 5. Main effect of water organic acid and monoglyceride supplementation on growth performance of nursery pigs[1]

Item	Control	Protaq[2]	SEM	*P* =
BW, lb				
d 0	11.5	11.5	0.21	0.943
d 7	12.6	12.9	0.22	0.001
d 21	24.6	24.4	0.45	0.478
d 42	52.1	52.1	0.82	0.924
d 0 to 7				
ADG, lb	0.16	0.20	0.010	< 0.001
ADFI, lb	0.31	0.30	0.007	0.670
F/G	2.27	1.62	0.228	< 0.001
d 7 to 21				
ADG, lb	0.85	0.83	0.016	0.020
ADFI, lb	0.98	0.95	0.017	0.043
F/G	1.14	1.14	0.009	0.894
d 21 to 42				
ADG, lb	1.31	1.31	0.025	0.892
ADFI, lb	1.93	1.94	0.055	0.717
F/G	1.47	1.48	0.015	0.641
d 0 to 42				
ADG, lb	0.95	0.95	0.018	0.689
ADFI, lb	1.32	1.32	0.032	0.830
F/G	1.39	1.38	0.008	0.277
Removals, %	5.6	3.9	0.74	0.450
Mortality, %	1.4	1.6	0.67	1.000
Total removals and mortality, %	7.1	5.6	0.80	0.531

[1]A total of 2,520 pigs (PIC 337 × 1050; initially 11.5 ± 0.21 lb) were used in a 42-d study.
[2]Protaq Bond 50 (liquid blend of organic acids and monoglycerides) was included in the water, via a water medicator, at a rate to reach a target pH of 4.0.

Table 6. Effects of diet and water monoglyceride and acid supplementation on fecal DM of nursery pigs[1]

Water:	Control			Protaq[2]				*P* =		
Item Diet:	Control	Acitra[3]	Acitra + EN[4]	Control	Acitra	Acitra + EN	SEM	Diet × water	Water	Diet
Fecal DM, %[5]										
d 10	24.8	24.3	22.5	22.0	25.9	24.4	1.08	0.107	0.773	0.208
d 24	19.3	19.0	17.5	18.1	20.2	19.4	0.71	0.095	0.278	0.246
d 42	21.2	23.0	22.7	19.6	22.7	21.4	0.77	0.015	0.076	0.005

[1]A total of 2,520 pigs (PIC 337 × 1050; initially 11.5 ± 0.22 lb) were used in a 42-d study with 30 pigs per pen and 14 replications per treatment across two rooms. On d 10, 24, and 42, fecal samples were collected from three pigs per pen for fecal DM analysis.
[2]Protaq Bond 50 (liquid blend of organic acids and monoglycerides) was included in the water, via a water medicator, at a rate to reach a target pH of 4.0.
[3]Acitra G20C (blend of organic acids) was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3.
[4]Acitra G20C was included at 0.3% in phase 1, 0.15% in phase 2, and 0% in phase 3. Entero-Nova 410C (blend of monoglycerides) was included at 0.3% in phases 1 and 2, and 0.1% in phase 3.
[5]Diet × water × day, $P = 0.289$; Diet × water, $P = 0.014$; Diet × day, $P = 0.212$; Water × day, $P = 0.051$; Day, $P < 0.001$; Diet, $P = 0.006$; Water, $P = 0.847$