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Influence of Herbal Active D on Nursery Pig Growth Performance

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Summary

A total of 2,268 pigs (L337 × 1050 PIC; initially 12.1 ± 0.39 lb) were used in a 42-d growth study to evaluate the effects of herbal active D on growth performance, bone characteristics, and serum parameters of nursery pigs. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 3 dietary treatments in a randomized complete block design. A total of 84 pens were used with 27 pigs per pen and 28 replications per treatment across 2 rooms. Pens were blocked by BW and weaning date. Dietary treatments were corn-soybean meal-based and fed in 3 phases. Treatment diets consisted of a control (contained 1,650 IU/kg of vitamin D_3), or control with the addition of 120 or 200 mg/kg of herbal active D (Phytobiotics, St. Louis, MO). At the end of the study, 10 pigs per treatment were euthanized and the right fibula, metacarpal, 2nd rib, and 10th rib were collected to determine bone density, bone breaking strength, and percentage bone ash by utilizing the de-fatted processing method. Overall (d 0 to 42), there was a marginally significant ($P = 0.067$) worsening of feed efficiency as inclusion of herbal active D increased but no effect ($P > 0.10$) on final BW, overall ADG, ADFI, or mortality. There was a bone × treatment interaction for bone density, where increasing herbal active D increased bone density for the 2nd rib ($P = 0.012$), but there was no difference between treatments for other bones ($P > 0.10$). For bone breaking strength and bone ash, there was no evidence ($P > 0.10$) of an interaction. For bone breaking strength, the metacarpal had greater breaking strength ($P < 0.001$) compared to all other bones, followed by the fibula and 10th rib, with the 2nd rib having the lowest bone breaking strength. For percentage bone ash, there was significant linear increase ($P = 0.026$) across all bones as herbal active D increased. For bone ash weight, the metacarpals and 10th ribs had the highest bone ash weight followed by the fibula, with 2nd rib having the lowest ($P < 0.05$) bone ash weight. Additionally, there was no difference ($P > 0.10$) across treatments for porcine circovirus type 2 S/P ratio, porcine reproductive and respiratory syndrome, *Mycoplasma hyopneumoniae*, 25(OH)D_3 status or circulating cytokine concentrations except for IL-8 concentrations which increased linearly ($P = 0.027$) as herbal active D increased. However, a day effect was observed ($P < 0.001$) with higher values for antibodies and cytokine concentrations on d 21 compared to d 42, except for IL-1ra and IL-8 having no significant ($P > 0.10$) day effect. In summary, herbal active D inclusion had minimal impact on growth or serum parameters; however, herbal active D increased percentage bone ash.

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Introduction

Vitamin D is a lipophilic vitamin that is required for growth, bone development and mineralization, and immune function. The two major forms of vitamin D are ergocalciferol (vitamin D_2) which is synthesized in plants, and cholecalciferol (vitamin D_3) which can be synthesized in the skin of animals and humans.[2]

Vitamin D_3 must be activated through a two-step hydroxylation process. After absorption in the small intestine, vitamin D_3 is stored in the liver where it is hydroxylated to produce 25-hydroxyvitamin D_3 [25(OH)D_3] which is vitamin D's major circulating metabolite.[3] After hydroxylation in the liver, 25(OH)D_3 is transported to the kidney and undergoes a second hydroxylation process to become 1,25-dihydroxicholecalciferol [1,25(OH)D_3] which is the most active form of vitamin D in the body.[4] However, under health challenges, low intake situations, or situations where liver or kidney conversions are less than sufficient, direct supplementation of 1,25(OH)D_3 may be beneficial.

In recent years, experiments have been conducted with different dietary vitamin D and 25(OH)D_3 inclusion rates.[5] However, neither of these additions increase the concentration of serum 1,25(OH)D_3. Direct addition of 1,25(OH)D_3 will provide the active form to the pig. Therefore, the objective of this study was to determine the response to herbal active D (Phytobiotics, St. Louis, MO) on nursery pig growth performance, mortality, bone characteristics, and blood measurements.

Procedures

Animals and diets

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at New Horizon Farms nursery research facility located in Pipestone, MN. The experiment utilized two identical nursery rooms that were completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 6-hole, dry self-feeder and a pan waterer to provide *ad libitum* access to feed and water. Feed additions were accomplished using a robotic feeding system (FeedPro, FeedLogic Corp., Wilmar, MN).

A total of 2,268 pigs (L337 × 1050 PIC; initially 12.1 ± 0.39 lb) were used in a 42-d growth study to evaluate the effects of herbal active D on growth performance, bone characteristics, and serum parameters of nursery pigs. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 3 dietary treatments in a randomized complete block design. A total of 84 pens were used with 27 pigs per pen and 28 replications per treatment across 2 rooms. Pens were blocked by BW and weaning date.

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Dietary treatments were corn-soybean meal-based and fed in 3 phases. Treatment diets consisted of a control with vitamin D_3 inclusion of 1,650 IU/kg, or control with the addition of 120 or 200 mg/kg of herbal active D (Table 1; Phytobiotics, St. Louis, MO) in addition to the 1,650 IU/kg of vitamin D_3. Pens of pigs were weighed on d 0, 7, 14, 21, 28, 35, and 42 to determine ADG, ADFI, and feed efficiency.

Blood analysis

On d 21 and 42, 25 gilts per treatment were bled for determination of circulating cytokine concentrations, antibody titers, and vitamin D status. Blood was collected in tubes without anticoagulant to obtain serum. Blood was allowed to clot before centrifuging for 15 min at 1,500 × *g* to collect serum, and then samples were stored at -112°F until analyzed. Serum samples were sent to the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) for vitamin D status (d 42 samples only) and antibody titers of porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome (PRRS), and *Mycoplasma hyopneumoniae*, using an ELISA kit. Serum samples were also analyzed using a panel, testing for 13 cytokines (Eve Technologies, Calgary, AB Canada).

Bone characteristics

At the end of the study, 10 gilts per treatment (weighing closest to the average BW of the 10 pens) were euthanized and the right fibula, metacarpal, 2nd rib, and 10th rib were collected to determine bone density, bone breaking strength, and percentage bone ash by utilizing the de-fatted processing method. After removal, bones were stored at -4°F until analysis. Bone density was measured on each bone based on the Archimedes principle. Bone breaking strength was reported as the maximum compressive load on each bone via an Instron (Instron 5569, NV Lab, Norwood, MA). For the de-fatted processing method, bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were dried at 221°F for 7 d in a drying oven and then ashed in a muffle furnace at 1,112°F for 24 h to determine the percentage of ash relative to dried bone weight.

Statistical analysis

Growth performance, vitamin D status, and bone characteristic data were analyzed as a randomized complete block design. Pen was considered the experimental unit. Treatment was used as the fixed effect and block was used as the random effect. Initial pen average BW and date of entry into the facility were incorporated within the blocking structure. Linear and quadratic contrasts were evaluated within increasing herbal active D treatments considering the control diet as no added herbal active D. For bone characteristics, treatment, bone, and the associated interactions were considered fixed effects, with block and pig serving as random effects.

Antibody titers and cytokines were analyzed as repeated measures representing multiple observations on each pen over time and blood samples were collected on a specific day to account for duplicate analysis within a single assay. Treatment, day, and the associated interactions were considered fixed effects. A Log_2 transformation was used for PCV2 antibody titers. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results and Discussion

Growth performance

For phase 1 (d 0 to 7), a linear increase ($P = 0.030$; Table 2) in ADG and a marginally significant linear increase ($P \leq 0.056$) in d 7 BW and improvement in feed efficiency were observed as herbal active D increased. Treatment diets had no effect ($P > 0.10$) on ADFI.

For phase 2 (d 7 to 21), pigs fed increasing levels of herbal active D had a linear reduction ($P \leq 0.050$) in d 21 BW, ADG, and poorer feed efficiency. No statistical differences ($P > 0.10$) were observed in ADFI.

Overall (d 0 to 42), a marginally significant ($P = 0.067$) worsening of feed efficiency was observed as herbal active D levels increased. Additionally, treatment diets had no effect ($P > 0.10$) on final BW, ADG, or ADFI. No statistical differences ($P > 0.10$) in mortality, removals, or mortality of the removed pigs were observed.

Bone characteristics

For bone density, a linear herbal active D × bone interaction ($P = 0.021$; Table 3) was observed. There was a linear ($P = 0.012$) effect of herbal active D for the 2nd rib where bone density increased as herbal active D increased in the diet. There was no effect of active herbal D for any other bones for bone density ($P > 0.10$). A main effect of bone was observed with 10th ribs having the highest bone density ($P < 0.001$; Table 4).

For bone breaking strength, no herbal active D × bone interaction ($P > 0.10$) was observed. However, a main effect of bone ($P < 0.0001$) was observed with metacarpals having the highest values for breaking strength and 2nd ribs having the lowest values. Treatment diets had no effect ($P > 0.10$) on bone breaking strength.

For percentage bone ash, no herbal active D × bone interaction ($P > 0.10$) was observed. Bone ash increased linearly ($P = 0.026$) as herbal active D increased. Additionally, a main effect of bone was observed where fibulas had the greatest percentage bone ash, followed by the 10th rib, with the 2nd rib and metacarpal having the lowest ($P < 0.05$) percentage bone ash.

For bone ash weight, no herbal active D × bone interaction ($P > 0.10$) was observed. A main effect of bone ($P < 0.0001$) was observed with metacarpals and 10th ribs having the greatest bone ash weight followed by the fibula, with the 2nd rib having the lowest ($P < 0.05$) bone ash weight. Treatment diets had no effect ($P > 0.10$) on bone ash weight.

Blood analysis

No treatment × day interactions were observed ($P > 0.10$; Table 5) for any of the blood measurements collected on d 21 and 42. Treatment diets had no effect ($P > 0.10$) on PCV2, PRRS, and *Mycoplasma hyopneumoniae* antibody titers, vitamin D status, or most circulating cytokine concentrations. However, a linear reduction ($P = 0.037$) in IL-8 concentrations as herbal active D increased was observed. Furthermore, a main effect of day was observed where pigs had increased ($P < 0.001$) PCV2, PRRS, and *Mycoplasma hyopneumoniae* antibody titers, and lower circulating cytokine concentra-

tions on d 42 compared to d 21. A main effect of day was not observed for IL-1ra and IL-8 ($P \geq 0.957$).

In summary, herbal active D inclusion had minimal impact on growth performance or serum parameters; however, herbal active D increased percentage bone ash in nursery pigs.

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Table 1. Diet composition (as-fed basis)

Ingredient, %	Phase 1[1]	Phase 2[2]	Phase 3[3]
Corn	38.28	54.07	57.10
Soybean meal, dehulled	25.14	26.13	29.57
DDGS	---	---	10.00
Whey powder	12.50	10.00	---
Whey permeate	11.25	---	---
Microbial-enhanced soy protein[4]	6.25	5.00	---
Choice white grease	---	1.00	---
Soybean oil	3.00	---	---
Calcium carbonate	0.62	0.70	0.90
Monocalcium P	0.88	0.95	0.65
Salt	0.35	0.60	0.50
L-Lys-HCl	0.40	0.38	0.45
DL-Met	0.25	0.20	0.12
L-Thr	0.18	0.19	0.19
L-Trp	0.02	0.03	0.03
L-Val	0.10	0.07	0.05
Vitamin and trace mineral premixes[5]	0.40	0.45	0.45
Zinc oxide	0.39	0.25	---
Herbal active D[6]	+/-	+/-	+/-
Total	100	100	100

continued

Table 1. Diet composition (as-fed basis)

Ingredient, %	Phase 1[1]	Phase 2[2]	Phase 3[3]
Calculated analysis			
SID AA, %			
Lys	1.40	1.35	1.30
Ile:Lys	61	62	59
Leu:Lys	114	122	130
Met:Lys	38	37	33
Met and Cys:Lys	56	56	56
Thr:Lys	64	64	63
Trp:Lys	20.4	20.2	19.2
Val:Lys	77	71	70
Total Lys, %	1.55	1.50	1.47
ME, kcal/lb	1,581	1,514	1,478
NE, kcal/lb	1,185	1,121	1,083
SID Lys:ME, g/Mcal	5.36	5.46	5.44
CP, %[7]	22.1	22.3	22.3
Ca, %	0.68	0.68	0.65
STTD P, %	0.54	0.52	0.45

[1]Phase 1 diets were fed from approximately d 0 to 7 (12 to 13 lb).
[2]Phase 2 diets were fed from approximately d 7 to 21 (13 to 22 lb).
[3]Phase 3 diets were fed from approximately d 21 to 42 (22 to 44 lb).
[4]Me-Pro, Prairie AquaTech, Brookings, SD.
[5]Ronozyme HiPhos (DSM, Parsippany, NJ) included in phase 1 diets at 1,250 FTU/kg provided an estimated release of 0.13% STTD P. Optiphos 2,500 G (Huvepharma; Peachtree City, GA) included in phase 2 and 3 diets provided an estimated release of 0.13% STTD P with 1,251 FTU/kg.
[6]Herbal active D (Phytobiotics, St. Louis, MO) was diluted with wheat middlings, such that a 1.2 lb/ton inclusion provided 120 mg/kg in the final diet and a 2 lb/ton inclusion provided 200 mg/kg of herbal active D.
[7]CP = crude protein.

Table 2. Influence of herbal active D on nursery pig growth performance[1]

Item	Control	120 mg/kg herbal active D[2]	200 mg/kg herbal active D[2]	SEM	*P* = Linear	*P* = Quadratic
BW, lb						
d 0	12.2	12.1	12.2	0.39	0.924	0.639
d 7	12.9	13.0	13.1	0.39	0.056	0.855
d 21	22.6	22.4	22.2	0.60	0.050	0.615
d 42	44.3	44.3	43.4	0.92	0.115	0.300
d 0 to 7 (phase 1)						
ADG, lb	0.10	0.12	0.13	0.011	0.030	0.921
ADFI, lb	0.25	0.25	0.26	0.011	0.224	0.731
F/G	2.53	2.15	2.07	---	---	---
G:F	0.395	0.466	0.482	0.0363	0.048	0.626
d 7 to 21 (phase 2)						
ADG, lb	0.65[a]	0.64[a,b]	0.62[b]	0.018	0.015	0.361
ADFI, lb	0.78	0.79	0.79	0.017	0.448	0.641
F/G	1.20[a]	1.24[b]	1.28[c]	---	---	---
G:F	0.835[a]	0.809[b]	0.783[c]	0.0120	< 0.001	0.536
d 21 to 42 (phase 3)						
ADG, lb	1.01	1.01	0.99	0.022	0.304	0.420
ADFI, lb	1.53	1.54	1.50	0.031	0.306	0.371
F/G	1.52	1.52	1.52	---	---	---
G:F	0.660	0.660	0.659	0.0064	0.825	0.912
d 0 to 42						
ADG, lb	0.72	0.73	0.71	0.016	0.359	0.289
ADFI, lb	1.04	1.05	1.04	0.020	0.891	0.352
F/G	1.44	1.45	1.47	---	---	---
G:F	0.693	0.689	0.681	0.0051	0.067	0.589
Removals, %	11.87	9.90	10.03	1.331	0.221	0.545
Mortality, %	3.69	2.90	3.29	0.705	0.613	0.493
Total mortality, %[3]	5.09	4.32	4.07	0.896	0.322	0.889
Total removals and mortality, %	15.54	12.77	13.30	1.528	0.177	0.371

[1]A total of 2,268 pigs (initially 12.2 ± 0.39 lb) were used with 27 pigs per pen and 28 replications per treatment. Treatment diets were fed in all 3 phases. F/G values were calculated using the inverse of the G:F values.
[2]Phytobiotics, St. Louis, MO.
[3]Percentage of pigs that died in original pen or hospital pen after being removed.

Table 3. Influence of herbal active D on nursery pig bone characteristics[1]

Item	Control	120 mg/kg herbal active D[2]	200 mg/kg herbal active D[2]	SEM
Bone density, g/mL[3]				
Fibula	1.28	1.29	1.28	0.009
2nd rib	1.26	1.28	1.29	
10th rib	1.29	1.30	1.29	
Metacarpal	1.22	1.21	1.21	
Bone breaking strength, lb[4]				
Fibula	34.3	32.8	39.0	3.30
2nd rib	13.0	14.9	13.7	
10th rib	29.4	30.4	33.0	
Metacarpal	88.4	85.9	85.8	
Bone ash, %[5]				
Fibula	63.1	63.7	63.5	0.43
2nd rib	58.3	59.1	59.1	
10th rib	59.0	60.5	60.5	
Metacarpal	58.4	58.6	59.4	
Bone ash, g[6]				
Fibula	1.02	1.03	1.14	0.078
2nd rib	0.77	0.83	0.82	
10th rib	1.45	1.50	1.54	
Metacarpal	1.50	1.51	1.55	

[1]A total of 2,268 pigs (initially 12.2 ± 0.39 lb) were used with 27 pigs per pen and 28 replications per treatment. Per treatment, 10 pigs were euthanized and the right metacarpal, fibula, 2nd rib, and 10th ribs were collected to determine bone ash weight and percentage bone ash utilizing the de-fatted processing method. Bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were dried at 221°F for 7 d in a drying oven and then ashed in a muffle furnace at 1,112°F for 24 h.

[2]Phytobiotics, St. Louis, MO.

[3]Bone density was measured on each bone based on the Archimedes principle. Linear herbal active D × bone interaction, $P = 0.021$. Linear effect of herbal active D for 2nd rib, $P = 0.012$. Linear effect of herbal active D for all other bones, $P > 0.10$. Main effect of bone, $P < 0.0001$.

[4]Bone breaking strength is reported as the maximum compressive load on each bone via an Instron (Instron 5569, NV Lab, Norwood, MA). Linear and quadratic herbal active D × bone interaction, $P > 0.10$. Main effect of herbal active D, $P > 0.10$. Main effect of bone, $P < 0.0001$.

[5]Bone ash was measured on each bone utilizing the de-fatted processing method. Linear and quadratic herbal active D × bone interaction, $P > 0.10$. Linear effect of herbal active D, linear $P = 0.030$. Main effect of bone, $P < 0.0001$.

[6]Bone ash weight was measured on each bone utilizing the de-fatted processing method. Linear and quadratic effect of herbal active D, $P > 0.10$. Main effect of bone, $P < 0.0001$.

Table 4. Main effects of herbal active D on nursery pig bone characteristics[1]

	Treatment				Bone				
Item	Control	120 mg/kg herbal active D[2]	200 mg/kg herbal active D[2]	SEM	Fibula	2nd rib	10th rib	Metacarpal	SEM
Bone density, g/mL[3]	1.26	1.27	1.27	0.007	1.286[a,b]	1.276[b]	1.292[a]	1.216[c]	0.0056
Bone breaking strength, lb[4]	41.3	41.0	42.8	2.28	35.3[b]	13.8[c]	30.9[b]	86.7[a]	2.37
Bone ash, %[5]	59.7	60.5	60.6	0.30	63.4[a]	58.8[c]	60.0[b]	58.8[c]	0.26
Bone ash, g[6]	1.19	1.22	1.26	0.071	1.06[b]	0.81[c]	1.50[a]	1.52[a]	0.066

[1]A total of 2,268 pigs (initially 12.2 ± 0.39 lb) were used with 27 pigs per pen and 28 replications per treatment. Treatment diets were fed in all 3 phases. Per treatment, 10 pigs were euthanized and the right metacarpal, fibula, 2nd rib, and 10th ribs were collected to determine bone ash weight and percentage bone ash utilizing the de-fatted processing method. All bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 days to remove water and fat. Bones were then dried at 221°F (105°C) for 7 days and then ashed in a muffle furnace at 1112°F (600°C) for 24 h.

[2]Phytobiotics, St. Louis, MO.

[3]Bone density was measured on each bone based on Archimedes principle. Linear and quadratic effect of herbal active D, $P > 0.10$. Main effect of bone, $P < 0.0001$.

[4]Bone breaking strength is reported as the maximum compressive load on each bone via the Instron machine (Instron 5569, NV Lab, Norwood, MA). Linear and quadratic effect of herbal active D, $P > 0.10$. Main effect of bone, $P < 0.0001$.

[5]Bone ash was measured on each bone utilizing the de-fatted processing method. Linear effect of herbal active D, $P = 0.030$. Main effect of bone, $P < 0.0001$.

[6]Bone ash weight was measured on each bone utilizing the de-fatted processing method. Linear and quadratic effect of herbal active D, $P > 0.10$. Main effect of bone, $P < 0.0001$.

Table 5. Main effects of herbal active D on nursery pig serum parameters[1]

	Treatment				*P* =		Day			
Item	Control	120 mg/kg herbal active D[2]	200 mg/kg herbal active D[2]	SEM	Linear	Quadratic	21	42	SEM	*P* =
Porcine circovirus type 2										
Log_2 titer	7.88	7.76	7.68	0.160	0.374	0.996	7.16	8.38	0.142	< 0.001
S/P ratio	0.47	0.44	0.42	0.035	0.373	0.997	0.31	0.58	0.031	< 0.001
Porcine reproductive and respiratory syndrome										
S/P ratio	0.78	0.77	0.78	0.050	0.972	0.870	0.08	1.48	0.057	< 0.001
Mycoplasma hyopneumoniae										
S/P ratio	0.20	0.19	0.20	0.039	0.949	0.825	0.08	0.31	0.034	< 0.001
25-hydroxyvitamin D_3, ng/mL[3]	20.0	20.1	19.1	0.93	0.514	0.600	---	---	---	---
Cytokine										
GM-CSF, pg/mL	40	43	72	27.6	0.447	0.633	86	17	27.7	0.006
IFNγ, pg/mL	8,945	9,984	9,048	1,806.5	0.925	0.651	15,211	3,441	1,841.7	< 0.001
IL-1α, pg/mL	93	95	96	14.1	0.873	0.960	124	66	13.2	0.001
IL-1β, pg/mL	599	554	579	79.2	0.824	0.736	737	417	73.5	0.001
IL-1ra, pg/mL	1,201	1,356	1,213	102.7	0.824	0.247	1,253	1,261	97.0	0.957
IL-2, pg/mL	657	621	622	98.5	0.774	0.895	872.7	394	93.7	< 0.001
IL-4, pg/mL	3,239	2,929	2,939	543.6	0.677	0.845	4,542	1,529	540.6	< 0.001
IL-6, pg/mL	294	265	266	56.7	0.699	0.853	390	160	56.8	< 0.001
IL-8, pg/mL	365	329	189	64.1	0.037	0.315	311	278	54.2	0.473
IL-10, pg/mL	1,648	1,644	1,516	243.5	0.707	0.793	2,207	998	238.7	< 0.001
IL-12, pg/mL	1,127	1,192	1,124	68.5	0.954	0.430	934	1,361	70.3	< 0.001
IL-18, pg/mL	4,155	3,920	3,416	625.5	0.407	0.782	4,997	2,664	603.5	0.001
TNFα, pg/mL	159	211	178	58.5	0.759	0.572	317	48.5	64.2	< 0.001

[1]A total of 2,268 pigs (initially 12.2 ± 0.39 lb) were used with 27 pigs per pen and 28 replications per treatment. Serum samples were collected from 1 average weight gilt from 25 pens per treatment. The pig was identified and blood was collected from the same gilt on d 21 and 42. Antibody titers and vitamin D concentration were analyzed at the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA). Cytokine analysis was conducted at Eve Technologies (Calgary, AB Canada). No treatment × day interactions were observed, $P > 0.10$.

[2]Phytobiotics, St. Louis, MO.

[3]Samples were only analyzed on d 42.