The Effect of Paylean® and Dietary Crude Protein Level on Odor Production and Nutrient Concentration in Anaerobically Stored Pig Manure

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Abstract

The pressure on swine producers to produce more environmentally friendly pork has increased in recent years as swine production units have become larger. Large pork production units have the potential to concentrate large volumes of manure in small land areas. Decreased land area for manure application contributes to nutrient build up in soil, which can lead to contaminating waterways from runoff and leaching into ground or surface waters. Odors from pork production units have threatened their existence in some areas and have often led to nuisance lawsuits against production units. The majority of odors from swine units are created from the anaerobic degradation of excreted nitrogenous, sulfur and carbohydrate compounds in manure. Paylean (ractopamine hydrochloride) has been shown to enhance nitrogen utilization for lean growth in finishing pigs, but the effect of Paylean on nitrogen and phosphorus excretion and odorous compound production in stored manure is unknown. The objective of this study was to determine the production of odorous compounds and the concentration of nutrients in stored manure from pigs fed a Paylean diet at two levels of crude protein (CP) during the late finishing period.

Experimental Procedure

Twenty-four DeKalb EB crossbred barrows (Initial BW = 185 lbs.) were adapted to stainless steel metabolism stalls for total collection of feces and urine. Pigs were adapted to stalls for eight days followed by a ten-day acclimation to one of the four following treatments:

1. 13.8% CP, 0.80% Lys (control)
2. 13.8% CP, 1.10% Lys with 18 g/ton (20 ppm) Paylean (ractopamine-HCl)
3. 16.1% CP, 1.10% Lys
4. 16.1% CP, 1.10% Lys with 18 g/ton (20 ppm) Paylean (ractopamine-HCl)

A three-day total collection followed the diet acclimation period for use in simulated anaerobic pit systems. Pigs were fed at 2200 g/d and 4800 ml/d of water in four even feedings (700 h and 1600 h). Feces and non-acidified urine were collected twice daily prior to feeding. Urine was refrigerated at 38°F (3°C) and feces were stored at 50°F (10°C) during collection to maintain bacterial populations while minimizing bacterial activity. Feces and urine were blended based on the dry matter (DM) of feces to obtain a final slurried mixture of 7.5% DM. Manure from two pigs per treatment was paired for the slurry mixtures to obtain three pooled replicate samples per treatment. Simulated anaerobic pits were set up in four-liter glass jars using two liters of slurry from the last day of collection as the initial inoculum. Paired samples were used in duplicate jars for a total of 23 jars (n = 5, 6, 6, and 6 for diets 1, 2, 3, and 4; respectively). Additional slurry of each treatment was frozen in 38 mL aliquots and added to the jars three times a week (Mon., Wed., and Fri.). Jars were capped and air was pumped across the headspace of each jar at 200 cc/minute. Simulated pits were maintained for 64 days. Olfactometry air samples, total volatile fatty acids (VFAs), gas chromatography fibers, and mixed slurry samples were taken from each jar at d 0, 17, 35, and 64. Air samples were collected in 10 L Tedlar bags

and analyzed by an eight-person olfactometry panel using the St. Croix triangular force choice olfactometer. Volatile fatty acids were extracted with 25% metaphosphoric acid from the slurry samples and analyzed by gas chromatography for acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid. Fibers were used to absorb gaseous compounds from headspace air and analyzed directly using gas chromatography-mass spectrophotometry to determine concentration of odorous compounds. Slurry samples were analyzed for ammonium nitrogen and total nitrogen using micro Kjeldahl techniques and a colorimetric procedure was used to determine total phosphorus. Dry matter (DM) of slurry samples was determined after drying at 203°F (95°C) for 24 hrs and pH was measured using a calibrated glass electrode pH meter.

Data were analyzed using the GLM procedure of SAS for treatment effects and means were separated using probability of difference at the $P < .05$ level.

Results and Discussion

The initial average slurry DM for the incubation trial was 8.20%, 7.94%, 8.32%, and 7.67% for the 13.8% CP control, 16.1% CP without Paylean, 16.1% CP with Paylean, and 13.8% CP with Paylean diets, respectively (Table 1). Dry matter was not different between treatments at d 0 while the 16.1% CP diet with Paylean had 9%, 6%, and 10% more DM than the control treatment at d 17, 35, and 64, respectively ($P < .05$). The addition of slurry to jars three times a week was intended to maintain approximately 7-8% slurry DM to simulate manure addition to typical commercial pits and provide nutrients for continual bacterial activity. The general increase in DM at d 17, 35, and 64 seen with the addition of Paylean to the diet, relative to the respective control, may be due to decreased bacterial populations or decreased bacterial activity which may have resulted from limited nutrient additions or decreased pH also observed in incubations containing the Paylean treatments.

Slurry pH (Table 2) was reduced 12-16% ($P < .05$) and 6-7% for the 13.8% CP diet with Paylean and the 16.1% CP diet with Paylean, respectively, compared to similar CP diets without Paylean at all time points during the incubation. The 13.8% CP diet with Paylean had 12%, 8%, 7%, and 8% lower pH values at d 0, 17, 35, and 64, respectively, compared to the 16.1% CP diet with Paylean. The addition of Paylean to the 16.1% CP diet resulted in a 5% ($P < .05$) decrease in slurry pH when compared to the standard 13.8% CP industry diet. The lower slurry pH values occurring with the addition of Paylean are probably due to decreased urine pH. The lower urine pH is a result of less urea nitrogen excretion in urine from more efficient retention of nitrogen in pigs fed Paylean.

At the four time points, manure slurry ammonium nitrogen (Table 3) was reduced 29-32% and 21-47% ($P < .05$) from pigs fed Paylean at 16.1% CP and 13.8% CP, respectively, compared to similar CP diets without Paylean. Ammonium nitrogen was reduced 35%, 29%, 21%, and 14% ($P < .05$) for the 13.8% CP diet with Paylean compared to the 16.1% CP diet with Paylean at d 0, 17, 35, and 64, respectively. The 16.1% CP diet with no additional Paylean had ammonium nitrogen levels 16-56% ($P < .05$) higher than any of the other treatments across all time points. The lower initial ammonium nitrogen levels may in a small part be due to better nitrogen absorption in the small intestine and retention in lean tissue with the addition of Paylean which reduced nitrogen excretion in urine and shifted a greater percentage of nitrogen to feces. The resulting lower ammonium nitrogen levels at d 17, 35, and 64 with the Paylean diets are most likely a result of the lower ammonium nitrogen level in the initial slurry which was used to make aliquots for slurry addition throughout the trial. Additionally, the lower pH of slurry from pigs fed Paylean or less bacterial activity in the incubation flasks may have contributed to the reduction of slurry ammonium nitrogen values over time.

Total nitrogen (Table 4) was similarly reduced 24-28% and 28-39% ($P < .05$) from pigs fed Paylean at 16.1% CP and 13.8% CP, respectively, compared to similar CP diets without Paylean at the four time points. Total nitrogen was 25%, 22%, 19%, and 19% lower ($P < .05$) for the 13.8% CP diet with Paylean than the 16.1% CP diet with Paylean at d 0, 17, 35, and 64, respectively. The 16.1% CP diet had higher total nitrogen ($P < .05$) at all time points compared to the other three diets. Total nitrogen was reduced 13% and 11% ($P < .05$) at d 35 and 64, respectively, when comparing the 16.1% CP diet with Paylean to the standard industry 13.8% CP diet. This indicates that less nitrogen was excreted with Paylean addition to the low CP diets.

Total phosphorus (Table 5) was initially 12% and 9% lower ($P < .05$) in the 13.8% CP and 16.1% CP diets with Paylean, respectively, compared to similar CP treatments without Paylean. At d 17, the addition of Paylean to the 16.1% CP diet reduced total phosphorus levels 9% ($P < .05$) compared to a similar diet with no Paylean, while the addition of Paylean to the 13.8% CP diet only tended to reduce total phosphorus levels ($P < .10$) compared to the similar diet with no Paylean. A similar pattern was shown at d 35 with numerically lower total phosphorus levels with the addition of Paylean to either CP level in the diet, but reductions were not statistically different. At d 64, total phosphorus was 7% and 9% lower ($P < .05$) with Paylean addition to the 13.8% CP and 16.1% CP compared to similar diets without Paylean, respectively. Total phosphorus was reduced 8% ($P < .05$) at d 17 and 35 when the 13.8% CP industry diet was compared to the 16.1% CP diet with Paylean. Total phosphorus reductions may primarily be attributed to a greater phosphorus demand for increased lean deposition while feeding Paylean with some reduction due to the increased energy demand for protein accretion due to the enhanced lean deposition.

Effects of dietary treatments on volatile fatty acid production and composition tended to vary at different time points during the trial. Total volatile fatty acid (VFA) production (Tables 6a-6d) was higher ($P < .05$) for the 16.1% CP diet with no additional Paylean compared to a similar treatment with Paylean addition at d 17, 35, and 64. The 16.1% CP diet also had 21%, 28%, and 23% higher ($P < .05$) total VFA production at d 17, 35, and 64, respectively, than the 13.8% CP diet with Paylean. Principally, acetate was higher in the 16.1% CP diet with Paylean at d 0 causing that treatment to have the highest total VFA production. Acetate was reduced ($P < .05$) 17%, 24%, and 20% when Paylean was added to the 16.1% CP diet at d 17, 35, and 64, respectively, compared to the 16.1% CP diet without Paylean. Butyrate was reduced 50% ($P < .05$) when the 16.1% CP diet without Paylean was compared to the 16.1% and 13.8% CP diets with Paylean at d 64. Isobutyrate production was reduced ($P < .05$) 20% and 26%, 21% and 22%, and 29% and 36% at d 17, 35, and 64, respectively, when the 16.1% CP diet without Paylean was compared to the 16.1% CP and 13.8% CP diets with Paylean, respectively. Similarly, there was a 35%, 22%, and 41% reduction ($P < .05$) in isovalerate production when Paylean was added to the 16.1% CP diet compared to the same diet without Paylean at d 17, 35, and 64, respectively. At d 64, isovalerate production was also reduced ($P < .05$) 36% when the 13.8% CP diet was fed with Paylean compared to the same diet without Paylean. The reduction in branched chain volatile fatty acids is an indication that less dietary amino acids are reaching the colon suggesting efficient absorption in the small intestine and consequently less microbial breakdown of the amino acids occurring during storage. Results are reduced odorous compound production in the form of VFAs. The relative reduction in total VFAs over time when pigs were fed Paylean may also be indicative of less nutrients being available in those manure slurries and so there may be less microbial activity, or, conversely, the microbial population is using the VFAs as an energy substrate.

Headspace air was collected from each jar and used for an eight-person olfactometry analysis, which determines the detection threshold (DT) and recognition threshold (RT) of the odor in the air samples (Table 7). The detection threshold is defined as the amount (ppm) of

clean air needed to dilute the odorous compounds in the headspace air sample until odor is not recognizable. The recognition threshold is the amount of odorous air needed for the panelist to assign a description to the odor. The initial odor detection and recognition values were not different among treatments, although the Paylean containing dietary treatments were numerically lower. At d 64 the detection threshold was 51% higher and the recognition threshold was 52% higher ($P < .05$) for the 16.1% CP diet without Paylean compared to the 13.8% CP diet without Paylean. The detection threshold was 49% and 48% lower ($P < .05$) for the 13.8% CP and 16.1% CP diets with Paylean, respectively, compared to the 16.1% CP diet without Paylean at d 64. These lower odor detection and recognition thresholds are probably due to the decreased concentrations of ammonia, VFAs, and other potentially odorous compounds in the slurry from dietary treatments with Paylean addition.

Gas chromatography fiber data (Table 8) indicates at d 0 methylphenol was 56%, 72%, and 94% lower ($P < .05$) when the 13.8% CP diet without Paylean, the 16.1% CP diet with Paylean, and the 13.8% CP diet with Paylean are compared to the 16.1% CP diet without Paylean, respectively. At d 17, phenol tended to decrease ($P < .10$) when Paylean was added to the 13.8% CP diet compared to the similar treatment without Paylean while butanol tended to increase ($P < .10$) when Paylean was added to the 16.1% CP diet when compared to the same diet without Paylean. Nonocane tended to be lower ($P < .10$) at d 35 when Paylean was added to either the 13.8% or 16.1% CP diet.

Application

Odors and excretion of nutrients from swine are major concerns to pork producers. Any economical and reliable means to significantly reduce odors and nutrient excretion will be readily implemented by the industry. Paylean addition reduced manure total nitrogen and ammonia nitrogen while also reducing pH and VFA production. Paylean also proves to be effective at reducing total phosphorus in stored manure 9-12% and select odorous compound production 48-49%. The data presented here also indicates that choosing to feed Paylean to growing pigs at the recommended 16% CP level will significantly reduce pH, total nitrogen and total phosphorus when compared to the typical industry 13.8% CP diet. This indicates that the use of Paylean to enhance protein accretion may also be beneficial in reducing environmentally detrimental nutrient levels in stored manure and in reducing the odor generated during storage. This will enhance the producer's ability to apply manure at environmentally safe levels and also help prevent potential offensive odors burdening neighbors.

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Table 1. Slurry dry matter

Dietary CP	Paylean, 20 ppm	DM, %			
		d 0[1]	d 17	d 35	d 64
13.8	-	8.20[a]	7.57[b]	7.25[a]	7.09[a]
16.1	-	7.94[ab]	8.04[ac]	7.60[ab]	7.57[ab]
16.1	+	8.32[a]	8.30[a]	7.72[b]	7.86[b]
13.8	+	7.67[b]	7.69[bc]	7.40[ab]	7.48[ab]
CV		4.98	13.58	3.99	5.15

[ab] Differing superscripts within a column indicate significance at P < .05
[1] Day on trial

Table 2. Slurry pH

Dietary CP	Paylean, 20 ppm	PH			
		d 0[1]	d 17	d 35	d 64
13.8	-	8.77[a]	8.47[a]	8.55[a]	8.55[a]
16.1	-	8.95[a]	8.62[a]	8.67[a]	8.65[a]
16.1	+	8.40[b]	7.99[b]	8.07[b]	8.11[b]
13.8	+	7.38[c]	7.33[c]	7.54[c]	7.45[c]
CV		1.85	1.70	1.76	1.58

[abc] Differing superscripts within a column indicate significance at P < .05
[1] Day on trial

Table 3. Total slurry ammonium nitrogen

Dietary CP	Paylean, 20 ppm	Ammonium N, ppm			
		d 0[1]	d 17	d 35	d 64
13.8	-	5063[a]	4612[b]	4279[a]	3745[a]
16.1	-	6144[b]	5976[a]	5091[b]	4933[b]
16.1	+	4170[c]	4218[b]	3390[c]	3453[a c]
13.8	+	2706[d]	3004[c]	2691[d]	2954[c]
CV		11.98	7.06	10.67	11.07

[abcd] Differing superscripts within a column indicate significance at P < .05
[1] Day on trial

Table 4. Slurry total nitrogen

Dietary CP	Paylean, 20 ppm	Total N, ppm			
		d 0[1]	d 17	d 35	d 64
13.8	-	7360a	6352a	5826a	5501a
16.1	-	8330[b]	8123[b]	6618[b]	6080[b]
16.1	+	5976[c]	5938[a]	5051[c]	4908[c]
13.8	+	4498[d]	4604[c]	4069[d]	2034[d]
CV		8.21	7.02	6.69	6.69

[abcd] Differing superscripts within a column indicate significance at P < .05
[1] Day on trial

Table 5. Slurry total phosphorus

Dietary CP	Paylean, 20 ppm	Total P, ppm			
		d 0[1]	d 17	d 35	d 64
13.8	-	2717[ab]	2817[ab]	2797 [a]	2894 [ac]
16.1	-	2880a	2852a	2685 ab	2973 a
16.1	+	2633[b]	2599[c]	2558 [b]	2705 [bc]
13.8	+	2394[c]	2654[bc]	2686 [ab]	2686 [b]
CV		6.03	5.04	4.24	5.76

[abc] Differing superscripts within a column indicate significance at P < .05
[1] Day on trial

Table 6a. Slurry volatile fatty acid content at d 0 on trial

Dietary CP	Paylean, 20 ppm	Volatile Fatty Acids, mmol/L						
		Ac[d]	Pr	iBut	But	iVal	Val	Total
13.8	-	43.40[a]	5.38	.668	2.39	1.099	.385[a]	53.32[a]
16.1	-	41.83[a]	5.15	.641	2.37	.9755	.442[ab]	54.74[a]
16.1	+	52.39[b]	6.37	.657	2.85	1.062	.375[a]	61.97[b]
13.8	+	35.44[c]	5.87	.733	2.77	.9698	.567[b]	46.35[c]
CV		7.38	18.16	21.28	29.75	11.42	29.32	8.26

[abc] Differing superscripts within a column indicate significance at P < .05

[d]Ac = acetate, Pr = propionate, iBut = isobutyrate, But = butyrate, iVal = isovalerate, Val = valerate

Table 6b. Slurry volatile fatty acid content at d 17 on trial

Dietary CP	Paylean, 20 ppm	Volatile Fatty Acids, mmol/L						
		Ac[d]	Pr	iBut	But	iVal	Val	Total
13.8	-	85.32[ab]	10.12[a]	2.38[ab]	4.80[a]	2.58[a]	.324[a]	105.52[abc]
16.1	-	95.12[a]	10.40[a]	2.91[a]	5.35[ab]	4.00[b]	.355[a]	118.13[a]
16.1	+	78.97[b]	11.49[ab]	2.32[b]	5.42[ab]	2.59[a]	.347[a]	101.13[bc]
13.8	+	67.71[c]	13.52[b]	2.16[b]	6.56[b]	2.34[a]	.725[b]	93.02[c]
CV		10.73	17.95	19.33	23.58	20.39	39.60	12.44

[abc] Differing superscripts within a column indicate significance at P < .05

[d]Ac = acetate, Pr = propionate, iBut = isobutyrate, But = butyrate, iVal = isovalerate, Val = valerate

Table 6c. Slurry volatile fatty acid content at d 35 on trial

Dietary CP	Paylean, 20 ppm	Volatile Fatty Acids, mmol/L						
		Ac[d]	Pr	iBut	But	iVal	Val	Total
13.8	-	69.35[a]	9.64	2.22[ab]	5.14	2.06[ab]	.883	89.3[ab]
16.1	-	79.60[b]	10.17	2.59[a]	5.63	2.48[a]	1.32	101.8[a]
16.1	+	60.29[ac]	9.25	2.04[b]	4.89	1.93[ab]	.692	79.1[bc]
13.8	+	53.28[c]	10.18	2.02[b]	5.35	1.79[b]	.953	73.6[c]
CV		11.07	16.44	16.62	22.35	27.03	63.71	12.30

[abc] Differing superscripts within a column indicate significance at P < .05
[d]Ac = acetate, Pr = propionate, iBut = isobutyrate, But = butyrate, iVal = isovalerate, Val = valerate

Table 6d. Slurry volatile fatty acid content at d 64 on trial

Dietary CP	Paylean, 20 ppm	Volatile Fatty Acids, mmol/L						
		Ac[d]	Pr	iBut	But	iVal	Val	Total
13.8	-	64.08[ab]	10.06	2.16[ac]	3.63[a]	1.30[ab]	1.30[a]	82.26[ab]
16.1	-	69.50[a]	10.62	2.62[c]	5.03[b]	1.75[a]	1.81[b]	91.33[a]
16.1	+	55.94[b]	9.41	1.87[ab]	2.53[a]	1.04[bc]	1.26[a]	72.05[b]
13.8	+	54.37[b]	9.82	1.67[b]	2.52[a]	.829[c]	1.07[a]	70.27[b]
CV		16.68	18.12	17.81	35.87	29.71	19.88	16.08

[abc] Differing superscripts within a column indicate significance at P < .05
[d]Ac = acetate, Pr = propionate, iBut = isobutyrate, But = butyrate, iVal = isovalerate, Val = valerate

Table 7. Odor detection (DT) and recognition (RT) levels[1]

Dietary CP	Paylean, 20 ppm	d 0[2]		d 17		d 35		d 64	
		DT	RT	DT	RT	DT	RT	DT	RT
13.8	-	3697[a]	2041[a]	1874[a]	1060[a]	1869[a]	1156[a]	1005[a]	573[a]
16.1	-	3753[a]	2036[a]	1115[b]	567[b]	2942[c]	1688[b]	2053[b]	1204[b]
16.1	+	3574[a]	1975[a]	2052[a]	1224[a]	2727[a c]	1587[cb]	1076[a]	587[a]
13.8	+	3213[a]	1675[a]	1923[a]	1139[a]	4235[b]	2480[c]	1055[a]	587[a]
CV		58.31	61.97	75.52	82.34	71.69	66.82	65.66	78.38

[abc] Differing superscripts within a column indicate significance at P < .05

[1]DT=Detection threshold, measure of when panelist correctly identified which air stream has a different odor from the other two air streams; RT=Recognition threshold, measure of when panelist can describe the odor

[2]Day on trial

Table 8a. Selected odorous compounds in headspace air as detected by GC fibers at d 0 on trial

Dietary CP	Paylean, 20 ppm	Ac[c]	Phe	MPhe
13.8	-	42.08	.090	.103[a]
16.1	-	39.47	.181	.236[b]
16.1	+	12.04	.221	.065[a]
13.8	+	24.96	.127	.013[a]
CV		98.64	109.61	81.40

[ab] Differing superscripts within a column indicate significance at P < .05
[c]Ac = acetone, Phe = phenol, Mphe = methylphenol

Table 8b. Selected odorous compounds in headspace air as detected by GC fibers at d 17 on trial

Dietary CP	Paylean, 20 ppm	Ac[d]	Phe	But	Undec	Dodec
13.8	-	0	.030[a]	13.15[abc]	4.15	2.68
16.1	-	0	0[b]	0b	4.25	3.40
16.1	+	1.95	.015[ab]	152.55[c]	3.73	2.55
13.8	+	0	0[b]	8.16[ab]	3.07	1.86
CV		481.68	255.91	318.68	80.14	76.54

[abc] Differing superscripts within a column indicate significance at P < .10
[d]Ac = acetone, Phe = phenol, But = butane, Undec = undecane, Dodec = dodecane

Table 8c. Selected odorous compounds in headspace air as detected by GC fibers at d 35 on trial

Dietary CP	Paylean, 20 ppm	Ac[f]	But	Dec	Nono	Undec	Dodec
13.8	-	4.08	8.14	12.91	16.42[d]	43.75	14.66 [ac]
16.1	-	2.09	65.42	11.49	2.26[e]	39.94	4.67 [b]
16.1	+	4.96	24.72	9.43	0[e]	37.13	7.48 [ab]
13.8	+	2.28	21.92	16.30	8.24[de]	41.95	18.98 [c]
CV		231.31	263.67	51.94	181.74	65.97	66.64

[abc] Differing superscripts within a column indicate significance at P < .05
[de] Differing superscripts within a column indicate significance at P < .10
[f]Ac = acetone, But = butane, Dec = decane, Nono = nonocane, Undec = undecane, Dodec = dodecane

Table 8d. Selected odorous compounds in headspace air as detected by GC fibers at d 64 on trial

Dietary CP	Paylean, 20 ppm	Ac[a]	Dec	Nono	Undec	Dodec
13.8	-	10.52	24.91	54.55	71.36	27.81
16.1	-	1.97	17.47	60.94	47.22	19.35
16.1	+	24.37	24.37	51.15	48.55	21.51
13.8	+	20.52	20.52	47.83	64.24	22.65
CV		144.09	36.68	99.52	40.81	39.84

[a]Ac = acetone, Dec = decane, Nono = nonocane, Undec = undecane, Dodec = dodecane