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Effects of Storage Type and Inclusion of Sodium Propionate on Microbial Profile of Feed Ingredients Commonly Used in Pig Diets

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ABSTRACT

An experiment was conducted to investigate the effects of storage type and inclusion of antifungal agent (sodium propionate) on microbial profile of feed ingredients commonly used in pig diets. Total of six feed ingredients (corn, wheat, soybean meal (SBM), corn DDGS, fish meal and poultry by-products) were stored in granary or feed bin with or without antifungal agent (0.30% sodium propionate) for 8 weeks period and microbial profile were investigated at the beginning of the experiment (week 0) and at 2, 4, 6 and 8 weeks of storage. At the beginning of the experiment, microbial profile of all feed ingredients were not different (p>0.05) among ingredients stored in granary or feed bin with or without sodium propionate. Irrespective of storage type and addition of sodium propionate, salmonella was not detected in any of all feed ingredient during 8 weeks of storage period. Inclusion of sodium propionate reduced (p<0.05) populations of staphylococci in corn (2, 4, 6 and 8 weeks), wheat (8 week), SBM, DDGS, fish meal and poultry by-product (4, 6 and 8 weeks). Clostridia populations were reduced (p<0.05) in sodium propionate added corn, SBM, DDGS, fish meal, poultry by-products (4, 6 and 8 weeks) and wheat (6 and 8 weeks). Coliforms populations were reduced (p<0.05) in all sodium propionate added ingredients at 2, 4, 6 and 8 weeks of storage, whereas the inclusion of sodium propionate reduced total anaerobic bacteria in fish meal, (2, 4, 6 and 8 weeks), poultry by-product (2 and 4 weeks), SBM (4 and 6 weeks) and corn, wheat, DDGS (4, 6 and 8 weeks).Yeast and mold count were reduced (p<0.05) in wheat, SBM, DDGS, fish meal and poultry by-products (week 4, 6, and 8) and corn (week 6 and 8). However, storage type (granary vs. feed bin) had no effects (p>0.05) on populations of staphylococci, clostridia, coliforms, total bacterial count, yeast and mold counts during any storage period. The results obtained in the present study indicated that microbial profile of feed ingredients was not affected by storage type (granary vs. feed bin), but the inclusion of sodium propionate improved the microbial profile of all feed ingredients commonly used in pig diets.

Keywords: Feeders type, performance, digestibility, growing finishing pigs

Moisture content of dry feed ingredients and finished feeds is generally below 13 %. Therefore, when storing feed ingredients or formula feed in a cold environment at relative humidity of 70 %, molds are unlikely to generate. However, for a long-time storage or in the rainy or summer seasons, feed absorbs moisture, and water content in feed may exceed 15 %, depending on the storage condition. As water content in feed increases, molds in the feed activate and grow proliferously, causing loss of nutrients and deterioration of taste. Some mold species produce toxic substances such as aflatoxin and they will possibly cause adverse effects to livestock and poultry. Molds are more likely to generate at higher temperatures and on pellets and smaller-particle feed. The substance which has a function to suppress generation of molds is called mold inhibitor. Organic acids and their salts are used as preservatives for the preservation of feedstuffs (Lückstädt and Mellor, 2011). At the same time, they inhibit the growth of specific germs due to their antimicrobial properties.

Sodium or calcium propionate are added to stored grains to prevent further development of molds at levels of 0.2 to 0.3% to feeds with 14-17% moisture and 0.5 to 0.6% to feeds with 18-24% moisture (Tarr, 1996). Kishaba *et al.* (1968) studied 7 antifungal agents and demonstrated that no agent was effective enough against mold when used singly, but the proper combination of antifungal agent showed a positive interaction to control the mold. A positive effect of addition of propionic acid to high-moisture corn was also observed by Jones *et al.* (1970). In addition to preservative effects of propionic acid, Jones *et al.* (1970) also observed improved performance of animals. Therefore, the objectives of the present study was to investigate the effects of storage type (granary *vs.* feed bin) and inclusion of antifungal agent (sodium propionate) on microbial status of feed ingredients (corn, wheat, SBM, corn DDGS, fish meal and poultry by-products) commonly used in pigs diet.

MATERIALS AND METHODS

The protocol for the present experiment was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Republic of Korea.

Experimental procedure and sampling

Feed ingredients commonly used in pig's diet (viz. corn, wheat, soybean meal (SBM), corn DDGS, fishmeal and poultry by-products) were procured from commercial feed millers of Republic of Korea. At the beginning of the experiment, individual feed ingredients were tasted for its microbial profile and stored in granary or feed bin with or without antifungal agent (0.30% sodium propionate) for 8 weeks period. Microbial profile of individual ingredients was investigated at 2, 4, 6 and 8 weeks of storage.

The antifungal agent used in this study was a powder containing at least 98.5 % of sodium propionate (chemical formula: CH_3CH_2COONa) in the dried matter and showing a maximum of 4 % loss on drying. The solubility of sodium propionate in water was of 100 g/L at 25 ^{O}C. It was produced by reacting an aqueous solution of sodium hydroxide and propionic acid, followed by drying of the salt.

Microbial analysis

The microbial profile was analyzed by using culture technique as described previously (Choi *et al.,* 2009). One gram of feed ingredient sample was diluted with 9 ml of Buffer-fields phosphate buffer dilution solution, followed by further serial dilutions in Buffer-fields phosphate buffer dilution solution. The microbial groups analyzed were salmonella (Xyloselysine desoxycholate agar), staphylococci (Mannitol salt agar), clostridia (Tryptose sulphite cycloserine agar, Oxoid, Hampshire, UK), coliforms (Violet red bile agar, Difco Laboratories, Detroit, MI, USA), total bacterial count (plate count agar, Oxoid-Unipath), yeast and mold count (Potato dextrose agar, staphylococci (Mannitol salt agar). Duplicate plates were inoculated with 0.1 ml sample and incubated. The anaerobic conditions during the assay of anaerobic were created by using gas pack anaerobic system (BBL, No. 260678; Difco, Detroit,MI). The microbial populations were transformed (log10) before statistical analysis and expressed as log10 cfu/g of contents.

Statistical analysis

Data generated in the present study was analyzed as 2 x 2 factorial arrangements of treatments. The main effects of storage type, antifungal agents, and their interaction were determined by GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Probability values of $\leq$0.05 were considered significant. The microbial populations were transformed (log10) before statistical analysis and expressed as log10 cfu/g of contents.

RESULTS

Microbial profile of corn

Effects of storage type and antifungal agent on microbial profile of corn are presented in Table 1. At the beginning of the experiment, storage type and addition of sodium propionate had no effects on microbial profile of corn (p>0.05). Irrespective of storage type and addition of antifungal agent, salmonella was not detected in corn during 8 weeks of storage period. Inclusion of sodium propionate in corn reduced (p<0.05) populations of staphylococci and coliforms at 2, 4, 6 and 8 weeks of storage. Clostridia populations were reduced (p<0.05) in antifungal agent added corn, at 4, 6 and 8weeks. Populations of total anaerobic bacteria in corn were reduced at week 4, 6 and 8 of storage. Yeast and mold count were reduced in corn at the end of 6 and 8 weeks.

Table 1. Microbial profile of corn stored in granary or feed bin with or without sodium propionate

Item	Granary		Feed bin		SEM[1]	*p*—value[2]		
	Sodium propionate (+)	Sodium propionate (—)	Sodium propionate (+)	Sodium propionate (—)		S	A	S×A
Week 0								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.88	1.83	1.76	1.82	0.06	0.268	0.916	0.377
Clostridia	1.08	1.27	1.32	1.35	0.11	0.165	0.312	0.454
Coliforms	1.15	1.23	1.20	1.27	0.10	0.648	0.451	1.000
Total bacteria	4.75	4.83	4.88	4.85	0.09	0.399	0.820	0.515
Yeast and mold	3.35	3.24	3.42	3.39	0.12	0.374	0.590	0.763
Week 2								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.85	2.03	1.86	1.94	0.04	0.329	0.011	0.241
Clostridia	1.15	1.32	1.15	1.23	0.09	0.632	0.214	0.632
Coliforms	1.58	1.94	1.47	1.81	0.06	0.072	<0.001	0.872
Total bacteria	5.06	5.16	5.04	5.16	0.23	0.957	0.642	0.965
Yeast and mold	3.49	3.65	3.54	3.69	0.16	0.795	0.353	0.970
Week 4								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.99	2.29	1.92	2.24	0.09	0.489	0.004	0.918
Clostridia	1.32	1.55	1.27	1.47	0.09	0.522	0.045	0.857
Coliforms	1.90	2.65	1.92	2.39	0.10	0.236	<0.001	0.178
Total bacteria	5.25	5.73	5.21	5.76	0.23	0.978	0.042	0.876
Yeast and mold	3.64	4.14	3.67	4.10	0.25	0.992	0.084	0.897
Week 6								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.02	2.26	1.95	2.19	0.09	0.225	0.008	0.624
Clostridia	1.39	1.51	1.35	1.50	0.05	0.111	0.003	0.420
Coliforms	1.87	2.52	1.71	2.47	0.13	0.388	<0.001	0.697
Total bacteria	5.27	5.74	5.15	5.79	0.23	0.862	0.028	0.738
Yeast and mold	3.79	4.12	3.74	4.16	0.13	0.706	0.008	0.963
Week 8								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.05	2.33	1.97	2.19	0.11	0.378	0.049	0.778
Clostridia	1.42	1.57	1.27	1.59	0.09	0.482	0.025	0.362
Coliforms	1.64	2.56	1.61	2.40	0.19	0.613	0.001	0.747
Total bacteria	5.32	5.80	5.24	5.80	0.28	0.894	0.092	0.873
Yeast and mold	3.72	4.26	3.70	4.23	0.18	.878	0.010	0.978

[1] Standard error of means.

[2] S: main effect of storage type; A: main effect of antifungal agent; S×A: interaction between storage type and antifungal agent.

*Antifungal agent positive is recommended value.

Table 2. Microbial profile of wheat stored in granary or feed bin with or without sodium propionate

Item	Granary		Feed bin		SEM[1]	p—value[2]		
	Sodium propionate (+)	Sodium propionate (—)	Sodium propionate (+)	Sodium propionate (—)		S	A	S×A
Week 0								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.64	2.61	2.55	2.59	0.09	0.511	0.955	0.700
Clostridia	1.23	1.15	1.35	1.42	0.09	0.061	1.000	0.442
Coliforms	3.27	3.33	3.24	3.23	0.16	0.689	0.902	0.829
Total bacteria	3.59	3.53	3.58	3.66	0.17	0.730	0.954	0.677
Yeast and mold	3.23	3.42	3.36	3.35	0.12	0.807	0.467	0.398
Week 2								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.69	2.71	2.66	2.73	0.08	0.971	0.631	0.775
Clostridia	1.35	1.44	1.32	1.39	0.11	0.742	0.473	0.947
Coliforms	3.08	3.74	3.57	4.14	0.23	0.081	0.021	0.851
Total bacteria	4.19	4.53	4.06	4.25	0.20	0.342	0.211	0.723
Yeast and mold	3.35	3.72	3.40	3.81	0.29	0.805	0.199	0.946
Week 4								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.75	2.83	2.71	2.78	0.08	0.858	0.188	0.699
Clostridia	1.49	1.64	1.44	1.50	0.08	0.425	0.100	0.901
Coliforms	3.60	4.86	3.52	4.16	0.16	0.681	<0.001	0.957
Total bacteria	4.32	5.42	4.29	4.93	0.20	0.750	<0.001	0.641
Yeast and mold	3.45	4.11	3.49	3.85	0.24	0.768	0.014	0.887
Week 6								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.77	2.88	2.77	2.91	0.07	0.846	0.128	0.828
Clostridia	1.57	1.73	1.47	1.65	0.05	0.098	0.005	0.866
Coliforms	3.70	4.87	3.83	4.86	0.13	0.622	<0.001	0.609
Total bacteria	4.37	5.34	4.37	5.41	0.26	0.890	0.002	0.890
Yeast and mold	3.57	4.21	3.55	4.38	0.22	0.734	0.006	0.684
Week 8								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.78	2.93	2.80	2.96	0.07	0.694	0.045	0.921
Clostridia	1.59	1.81	1.45	1.72	0.08	0.166	0.010	0.759
Coliforms	3.61	4.93	3.74	4.73	0.19	0.839	<0.001	0.384
Total bacteria	4.26	5.32	4.25	5.30	0.22	0.964	<0.001	0.991
Yeast and mold	3.57	4.35	3.65	4.45	0.19	0.644	0.001	0.990

[1] Standard error of means.

[2] S: main effect of storage type; A: main effect of antifungal agent; S×A: interaction between storage type and antifungal agent.

*Antifungal agent positive is recommended value.

Table 3. Microbial profile of SBM stored in granary or feed bin with or without sodium propionate

Item	Granary		Feed bin		SEM[1]	p—value[2]		
	Sodium propionate (+)	Sodium propionate (—)	Sodium propionate (+)	Sodium propionate (—)		S	A	S×A
Week 0								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	4.81	4.83	4.93	4.92	0.09	0.277	0.930	0.875
Clostridia	3.32	3.33	3.31	3.30	0.05	0.695	0.982	0.872
Coliforms	2.35	2.30	2.33	2.34	0.04	0.878	0.647	0.414
Total bacteria	6.81	6.78	6.77	6.78	0.02	0.437	0.736	0.376
Yeast and mold	4.78	4.66	4.68	4.73	0.10	0.909	0.751	0.387
Week 2								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	4.87	4.94	4.83	4.88	0.08	0.557	0.457	0.942
Clostridia	3.33	3.40	3.30	3.38	0.07	0.737	0.291	1.000
Coliforms	2.42	2.66	2.24	2.59	0.10	0.211	0.012	0.581
Total bacteria	6.88	6.88	6.76	6.85	0.16	0.652	0.786	0.763
Yeast and mold	4.80	5.07	4.94	5.17	0.21	0.568	0.252	0.910
Week 4								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	4.92	5.25	4.94	5.04	0.09	0.317	0.031	0.233
Clostridia	3.37	3.56	3.37	3.52	0.05	0.696	0.007	0.696
Coliforms	2.44	3.08	2.29	3.06	0.08	0.328	<0.001	0.417
Total bacteria	6.85	7.14	6.74	7.17	0.19	0.831	0.080	0.703
Yeast and mold	4.76	5.49	4.71	5.55	0.19	0.995	0.001	0.778
Week 6								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	4.94	5.34	4.98	5.13	0.08	0.339	0.006	0.167
Clostridia	3.39	3.64	3.42	3.58	0.06	0.759	0.005	0.453
Coliforms	2.45	3.01	2.41	3.08	0.08	0.877	0.000	0.518
Total bacteria	6.87	7.13	6.82	7.17	0.14	0.957	0.044	0.774
Yeast and mold	4.80	5.39	4.72	5.56	0.17	0.757	0.001	0.465
Week 8								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	4.99	5.42	5.00	5.19	0.08	0.193	0.002	0.158
Clostridia	3.37	3.69	3.37	3.60	0.05	0.399	<0.001	0.349
Coliforms	2.34	2.98	2.38	3.05	0.07	0.462	<0.001	0.822
Total bacteria	6.92	7.23	6.86	7.26	0.18	0.946	0.075	0.799
Yeast and mold	4.79	5.35	4.74	5.47	0.24	0.896	0.021	0.725

[1] Standard error of means.

[2] S: main effect of storage type; A: main effect of antifungal agent; S×A: interaction between storage type and antifungal agent.

*Antifungal agent positive is recommended value.

Table 4. Microbial profile of corn DDGS stored in granary or feed bin with or without sodium propionate

Item	Granary Sodium propionate (+)	Granary Sodium propionate (—)	Feed bin Sodium propionate (+)	Feed bin Sodium propionate (—)	SEM[1]	*p*—value[2] S	*p*—value[2] A	*p*—value[2] S×A
Week 0								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.15	1.23	1.27	1.30	0.12	0.416	0.653	0.852
Clostridia	1.08	1.27	1.20	1.36	0.10	0.336	0.112	0.889
Coliforms	1.20	1.32	1.32	1.20	0.12	1.000	1.000	0.321
Total bacteria	3.27	3.35	3.27	3.27	0.11	0.735	0.735	0.735
Yeast and mold	2.76	2.78	2.80	2.77	0.07	0.811	0.956	0.755
Week 2								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.15	1.30	1.15	1.27	0.10	0.878	0.202	0.878
Clostridia	1.15	1.27	1.08	1.20	0.10	0.451	0.236	1.000
Coliforms	1.20	1.35	1.37	1.69	0.12	0.056	0.080	0.502
Total bacteria	3.57	3.87	3.52	3.88	0.28	0.938	0.267	0.924
Yeast and mold	2.58	2.58	2.47	2.96	0.19	0.490	0.204	0.212
Week 4								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.19	1.60	1.19	1.35	0.11	0.271	0.026	0.271
Clostridia	1.23	1.51	1.15	1.35	0.06	0.083	0.003	0.491
Coliforms	1.45	2.16	1.62	2.01	0.15	0.960	0.003	0.290
Total bacteria	3.75	4.34	3.81	4.57	0.23	0.545	0.014	0.727
Yeast and mold	2.50	3.30	2.58	3.31	0.15	0.741	<0.001	0.815
Week 6								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.27	1.79	1.30	1.48	0.10	0.169	0.004	0.099
Clostridia	1.27	1.65	1.20	1.47	0.11	0.279	0.015	0.640
Coliforms	1.32	2.37	1.27	2.04	0.13	0.162	<0.001	0.280
Total bacteria	3.71	4.47	3.73	4.55	0.32	0.879	0.031	0.939
Yeast and mold	2.43	3.23	2.84	3.37	0.26	0.323	0.027	0.620
Week 8								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.33	1.81	1.42	1.57	0.10	0.532	0.018	0.175
Clostridia	1.32	1.70	1.15	1.52	0.11	0.069	0.001	0.954
Coliforms	1.35	1.90	1.59	2.19	0.13	0.185	0.009	0.911
Total bacteria	3.51	4.04	3.66	4.51	0.32	0.128	0.003	0.419
Yeast and mold	2.30	3.15	2.37	3.12	0.26	0.848	<0.001	0.634

[1] Standard error of means.

[2] S: main effect of storage type; A: main effect of antifungal agent; S×A: interaction between storage type and antifungal agent.

*Antifungal agent positive is recommended value.

Table 5. Microbial profile of fish-meal stored in granary or feed bin with or without sodium propionate

Item	Granary		Feed bin		SEM[1]	*p*—value[2]		
	Sodium propionate (+)	Sodium propionate (—)	Sodium propionate (+)	Sodium propionate (—)		S	A	S×A
Week 0								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.69	1.68	1.81	1.75	0.11	0.416	0.759	0.835
Clostridia	1.72	1.75	1.63	1.63	0.11	0.366	0.886	0.903
Coliforms	1.15	1.08	1.12	1.15	0.09	0.814	0.814	0.585
Total bacteria	4.20	4.27	4.32	4.24	0.13	0.741	1.000	0.584
Yeast and mold	2.05	2.06	2.10	2.12	0.04	0.180	0.686	0.954
Week 2								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.74	1.85	1.77	1.76	0.08	0.698	0.566	0.469
Clostridia	1.75	1.85	1.73	1.81	0.07	0.720	0.251	0.932
Coliforms	1.32	1.74	1.54	1.94	0.10	0.050	0.001	0.900
Total bacteria	4.33	5.49	4.27	5.71	0.24	0.726	<0.001	0.565
Yeast and mold	2.14	2.27	2.12	2.29	0.12	0.992	0.230	0.860
Week 4								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.81	2.01	1.73	1.84	0.07	0.108	0.053	0.555
Clostridia	1.81	2.08	1.77	1.98	0.07	0.390	0.007	0.705
Coliforms	1.32	2.32	1.35	2.33	0.11	0.872	<0.001	0.908
Total bacteria	4.51	6.29	4.77	6.51	0.20	0.249	<0.001	0.917
Yeast and mold	2.18	2.47	2.09	2.45	0.15	0.699	0.055	0.809
Week 6								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.86	2.09	1.76	1.91	0.07	0.057	0.017	0.576
Clostridia	1.85	2.19	1.83	2.07	0.07	0.387	0.001	0.487
Coliforms	1.49	2.10	1.42	2.56	0.16	0.248	<0.001	0.124
Total bacteria	4.68	5.66	4.80	6.22	0.20	0.125	<0.001	0.295
Yeast and mold	2.27	2.54	2.03	2.54	0.13	0.368	0.012	0.377
Week 8								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	1.90	2.14	1.83	2.03	0.10	0.395	0.047	0.838
Clostridia	1.86	2.26	1.84	2.15	0.07	0.338	<0.001	0.499
Coliforms	1.27	2.35	1.37	2.29	0.19	0.903	<0.001	0.696
Total bacteria	4.76	5.32	4.81	5.63	0.26	0.498	0.020	0.622
Yeast and mold	2.32	2.60	2.09	2.57	0.18	0.481	0.052	0.586

[1] Standard error of means.

[2] S: main effect of storage type; A: main effect of antifungal agent; S×A: interaction between storage type and antifungal agent.

*Antifungal agent positive is recommended value.

Table 6. Microbial profile of poultry by-product stored in granary or feed bin with or without sodium propionate

Item	Granary		Feed bin		SEM[1]	p—value[2]		
	Sodium propionate (+)	Sodium propionate (—)	Sodium propionate (+)	Sodium propionate (—)		S	A	S×A
Week 0								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.34	2.36	2.31	2.24	0.06	0.217	0.697	0.467
Clostridia	1.20	1.24	1.25	1.32	0.14	0.640	0.692	0.942
Coliforms	1.23	1.15	1.27	1.20	0.11	0.700	0.523	1.000
Total bacteria	3.32	3.39	3.30	3.24	0.11	0.474	0.947	0.556
Yeast and mold	1.63	1.71	1.72	1.72	0.09	0.559	0.617	0.637
Week 2								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.39	2.41	2.36	2.39	0.03	0.495	0.495	0.748
Clostridia	1.20	1.30	1.20	1.27	0.12	0.898	0.449	0.898
Coliforms	1.20	1.56	1.35	1.79	0.13	0.159	0.007	0.763
Total bacteria	3.55	4.08	3.86	4.15	0.13	0.169	0.008	0.373
Yeast and mold	1.47	1.99	1.54	2.19	0.17	0.436	0.005	0.707
Week 4								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.43	2.61	2.42	2.51	0.04	0.189	0.003	0.233
Clostridia	1.32	1.60	1.27	1.50	0.10	0.476	0.025	0.785
Coliforms	1.15	2.24	1.60	2.49	0.19	0.093	<0.001	0.607
Total bacteria	3.94	4.63	4.17	4.80	0.16	0.241	0.002	0.870
Yeast and mold	1.52	2.16	1.69	2.40	0.11	0.098	<0.001	0.765
Week 6								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.46	2.69	2.49	2.59	0.04	0.475	0.003	0.177
Clostridia	1.36	1.69	1.30	1.62	0.08	0.411	0.001	0.949
Coliforms	1.20	2.21	1.53	2.32	0.16	0.214	<0.001	0.503
Total bacteria	3.92	4.20	4.03	4.20	0.11	0.644	0.069	0.675
Yeast and mold	1.89	2.66	1.61	2.78	0.11	0.462	<0.001	0.091
Week 8								
Salmonella	—	—	—	—	—	NS	NS	NS
Staphylococci	2.50	2.75	2.53	2.66	0.05	0.583	0.004	0.306
Clostridia	1.39	1.73	1.32	1.70	0.07	0.439	<0.001	0.794
Coliforms	1.27	2.59	1.39	2.48	0.12	0.968	<0.001	0.369
Total bacteria	3.85	4.16	4.04	4.50	0.18	0.177	0.060	0.695
Yeast and mold	1.78	2.53	1.42	2.73	0.14	0.571	<0.001	0.062

[1] Standard error of means.

[2] S: main effect of storage type; A: main effect of antifungal agent; S×A: interaction between storage type and antifungal agent.

*Antifungal agent positive is recommended value.

However, storage type (granary vs. feed bin) had no effects (p>0.05) on populations of staphylococci, clostridia, coliforms, total bacterial count, yeast and mold counts during any storage period. Also, there were no interaction (p>0.05) among storage type and sodium propionate addition in corn during any storage period.

Microbial profile of wheat

Storage type (granary vs. feed bin) had no effects (p>0.05) on populations of staphylococci, clostridia, coliforms, total bacterial count, yeast and mold counts of wheat during any storage period (Table 2). Also, storage type and sodium propionate addition had no effects (p>0.05) on microbial profile of wheat at the beginning of the experiment. Moreover, irrespective of storage type and addition of sodium propionate, salmonella was not detected in wheat during 8 weeks of storage period. Inclusion of sodium propionate reduced (p<0.05) populations of coliforms (2, 4, 6 and 8 weeks of storage), total anaerobic bacteria (4, 6 and 8 weeks) clostridia (week 6 and 8) and staphylococci. In addition, yeast and mold count in wheat were reduced (p<0.05) at the end of 4, 6, and 8 weeks of storage. Interaction among storage type and sodium propionate addition were not observed in this study.

Microbial profile of soybean meal (SBM)

During 8 weeks storage period, storage type (granary vs. feed bin) had no effects (p>0.05) on populations of staphylococci, clostridia, coliforms, total bacterial count, yeast and mold counts in soybean meal (Table 3). Microbial profile of was not different (p>0.05) among SBM stored in granary or feed bin with or without sodium propionate at d 0 of experiment. Salmonella was not detected in SBM during any storage period irrespective of storage type and addition of sodium propionate. Inclusion of sodium propionate in SBM reduced (p<0.05) populations of coliforms, total anaerobic bacteria (2, 4, 6 and 8 weeks) and staphylococci, clostridia (4, 6 and 8 weeks). There were no interaction (p>0.05) among storage type and sodium propionate addition in SBM during any studied period.

Microbial profile of corn DDGS

At the beginning of the experiment microbial profile of was not different (p>0.05) among corn DDGS stored in granary or feed bin with or without sodium propionate (Table 4). Irrespective of storage type and addition of sodium propionate, salmonella was not detected in corn DDGS during any studied period. At 4, 6 and 8 weeks, inclusion of sodium propionate reduced (p<0.05) populations of staphylococci, clostridia, coliforms and total anaerobic bacteria in corn DDGS. Moreover, yeast and mold count were reduced (p<0.05) in DDGS at week 4, 6, and 8 weeks of storage. However, storage type (granary vs. feed bin) had no effects (p>0.05) on populations of staphylococci, clostridia, coliforms, total bacterial count, yeast and mold counts during any storage period.

Microbial profile of fish meal

Storage type (granary vs. feed bin) had no effects (p>0.05) on populations of staphylococci, clostridia, coliforms, total bacterial count, yeast and mold counts of fish meal during any studied period (Table 5). At the beginning of the experiment microbial profile of was not different (p>0.05) among fish meal stored in granary or feed bin with or without sodium propionate. During 8 weeks of storage period, salmonella was not detected in fish meal stored in granary or feed bin with or without sodium propionate. Inclusion of sodium propionate reduced (p<0.05) populations of coliforms (2, 4, 6 and 8 weeks), staphylococci, clostridia, yeast and mold count (week 4, 6 and 8). There were no interaction (p>0.05) among storage type and sodium propionate addition in fish meal during any storage period.

Microbial profile of poultry by-product

At the beginning of the experiment microbial profile was not different (p>0.05) among of poultry by-product stored in granary or feed bin with or without sodium propionate (Table 6). Irrespective of storage type and addition of sodium propionate, salmonella was not detected in poultry by-product during 8 weeks of storage period. Inclusion of sodium propionate to poultry by-product reduced (p<0.05) populations of coliforms (2 and 4 weeks), staphylococci, clostridia, yeast and mold (4, 6 and 8 weeks). However, storage type (granary vs. feed bin) had no effects (p>0.05) on populations of staphylococci, clostridia, coliforms, total bacterial count, yeast and mold counts during any storage period. No interaction among storage type and sodium propionate addition were observed (p>0.05).

DISCUSSION

Aflatoxins, secondary metabolites produced by *Aspergillus* spp., are common contaminant of pigs feed under tropical and subtropical climates. Aflatoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus* are some of the toxic mycotoxins and aflatoxin B1 is the most toxic aflatoxin (Devegowda and Murthy, 2005). Like other non ruminant species, pigs are unable to efficiently metabolize aflatoxins, making them highly susceptible to aflatoxicosis. In pigs, severity of aflatoxin infection depends upon dose of ingestion through infected dietary ingredients. Previously it has been reported that addition of aflatoxin binder or mould inhibitors to contaminated diets can greatly reduce the bioavailability of toxins in the gastrointestinal tract (Carson and Smith, 1983; Phillips *et al.,* 1988). Therefore, the present experiment was conducted to investigate the effects of storage type and inclusion of antifungal agent on microbial profile of feed ingredients commonly used in pig diets.

Antifungal agents with varied modes of action have been used, especially to prevent mold growth on feed during storage and processing. Molds reduce palatability and can produce mycotoxins (aflatoxin and fusarium) in animal feeds. Organic acids have been used for decades in commercial compound feeds, mostly for feed preservation, for which formic and propionic acids and their salts (calcium and sodium propionate) are particularly effective (Lückstädt and Mellor, 2011). Organic acids and their salts are most commonly used feed preservatives or mold inhibitors in feed industry since decades. Organic acids are defined as feed additives with beneficial antifungal effects in feed (Dixon and Hamilton, 1981), but propionic acid also shows a strong antibacterial activity in contaminated feeds, especially against *salmonella spp.* (Khan and Katamay, 1969; Hinton and Linton, 1988; Izat *et al.,* 1990; McHan and Shotts, 1992; Thompson and Hinton, 1997). The overall result of current study indicated that addition of sodium propionate resulted into significant reduction in pathogenic bacteria (staphylococcus, clostridia, coliforms and total anaerobic bacteria) and yeast populations during 8 weeks for feed ingredients stored in granary or feed bin.

Organic acids exert their effects by different mode of actions. They are both bacteriostatic and bactericidal. As undissociated organic acids are lipophilic, they can cross the cell membrane of Gram negative bacteria, such as *Salmonella*. Once inside the cell, the higher cytosolic pH causes the acid to dissociate, releasing hydrogen ions, which consequently reduces the intracellular pH. Microbial metabolism is dependent on enzyme activity, which is depressed at lower pH. To redress the balance, the cell is forced to use energy to expel protons out across the membrane via the H+-ATPase pump to restore the cytoplasmic pH to normal. Over a period of exposure to organic acids, this can be sufficient to kill the cells of pathogenic microorganisms. Expelling protons also leads to an accumulation of acid anions in the cell (Lambert and Stratford, 1998), which inhibits intracellular metabolic reactions, including the synthesis of macromolecules, and disrupts internal membrane (Lückstädt and Mellor, 2011). It has been reported that propionic acids and their salts has been commonly used as mould inhibitor or antifungal agent in pigs diets (Strauss and Hayler, 2001). It has been reported that mold growth occurred very rapidly in diets with little or no antifungal agents (Ludemann et al., 1979; Holleley *et al.,* 2008). Roeder et al. (2009) conducted an experiment by the utilization of microbial inhibitors in artificial diet to observe microbial colonization, fungi and mold growth in the diet and get some encouraging results. Antifungal agent are usually expected to be stable without reacting or binding with other dietary components, or being degraded by the process of heat treatment (Hedin *et al.,* 1974). However, Brock and Buckel (2004) found that sodium propionate inhibits growth of *Aspergillus nidulans* on glucose but not on acetate. Ludemann *et al.* (1979) observed that the effects of antifungal agents vary as per storage duration. He reported that most of antifungal agents are not effective for more than 2 weeks. In the present study, most prominent results of addition of sodium propionate to all feed ingredients occurred during 4 , 6 and 8 weeks of storage.

In the present study, inclusion of sodium propionate resulted into reduction in populations of Staphylococci, coliforms and clostridium spp after 4 weeks of storage and inhibition of mold growth after 6 weeks of preservation in most feed ingredients irrespective of storage type. Present findings are consistent with Hugo *et al.* (2015) who observed that lactic acid and lactates were effective against bacteria, while sodium propionate is effective against moulds. Kishaba *et al.* (1968) studied 7 antifungal agents and demonstrated that no agent was effective enough against mold when used singly, but the proper combination of antifungal agent showed a positive interaction to control the mold. It has been reported that organic acids are not able to reduce the population of pathogens after 8 weeks of storage and the pathogens can be recovered after 3 to 18 months after the addition of organic acids in the feed (Williams and Benson, 1978; Davies and Wray, 1996; Ha *et al.,* 1998 a, b).

CONCLUSION

In conclusion, the results obtained in the present study indicated that microbial profile of feed ingredients was not affected by storage type (granary vs. feed bin), but the inclusion of antifungal agent improved the microbial profile of all feed ingredients commonly used in pig diets.

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