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Stability of commercially available lactobacillus products

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Stability of Commercially Available Lactobacillus Products

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Summary

A study was conducted to determine the viability of three commercially available lactobacillus products in nonmedicated and medicated (lincomycin at 100 grams per ton) swine feed in several different environments over a 3-month period. Each product was stored in five environments: refrigeration (40°F), room temperature (70°F) in sealed container, room temperature in unsealed container, swine nursery (90°F) in a sealed container, and swine nursery in an unsealed container. Feed samples were counted for lactobacillus at weeks 0, 1, 3, 5, 7, and 12 with four replications. The three commercial products differed in stability while maintained in the various environmental conditions. Refrigerated storage prolonged (P<.05) stability of all products. Stability of products stored at room temperature was greater than those stored in the nursery (P<.05). Medication had a slight adverse affect on the product stability. There were no differences between samples stored in sealed or open containers. These results demonstrate that the stability of viable lactobacillus products can be influenced by storage environment, length of storage, and type of medication in the feed.

Introduction

In the past few years numerous lactobacillus products have been merchandized to improve performance of pigs and reduce the incidence of scours by suppressing E. coli populations. The stability of these viable cultures is not well established when stored under conventional systems. It is well documented that temperature, humidity, change in pH, and various antibiotics will decrease the viability of lactobacillus cultures. In conventional feed storage systems all four factors may be involved. Therefore, the objective of this study was to evaluate the stability of commercially available lactobacillus products stored in several environmental conditions.

Experimental Procedure

The three commercial lactobacillus products selected for this study were provided by: Pioneer Hibrid International (Probios®), Great Lakes Biochemical (Lact-A-Bac®), and Fermented Products. All three products were added to a conventional starter diet containing 20% dried whey and 1.2% lysine. Products were added at the rate of one million to a billion organisms per gram of complete feed. Products were mixed into diets containing no medication or lincomycin at 100 grams per ton of feed. The non-medicated and medicated diets were stored in five environmental conditions: refrigeration (40°F), room temperature (70°F) in a sealed container, room temperature in an open container, nursery (90°F) in a sealed container, and in a nursery in an open

[1]Appreciation is expressed to the commercial companies for donating product and partial financing for this trial.

container. These five environments were selected to represent the temperature range differences that are commonly associated with the storage of viable microbial cultures.

After the cultures were mixed into the feed, a gram sample was taken for enumeration of lactobacillus organisms before storage (week 0) in the various environments. The counting procedure was repeated at 1, 3, 5, 7, and 12 after the initiation of the trial. Organisms were counted using MRS agar incubated anaerobically for 48 hours. Number of organisms were counted and expressed as a log of the colony-forming units (CFU) per gram of feed with four replications.

An antibiotic sensitivity test was conducted with the three products (Table 1). Two colony types (white and ivory) were isolated from all the commercial products. Commonly used antibiotics were tested to determine the extent of organism growth. Neither colony type would grow in antibiotic combination of chlorotetracycline, sulfamethazine, and penicillin. The antibiotic sensitivity data suggest that both colony types are sensitive to tetracycline and penicillin but are resistant to sulfa. It is important that the selected antibiotic not reduce total microbial growth. Therefore, lincomycin was selected as the antibiotic since lactobacillus organisms were resistant to it. Earlier studies have suggested that lactobacillus cultures in combination with lincomycin in starter pig diets may have an additive effect.

Results and Discussion

The effect of medication on the number of organisms per gram of feed is shown in Table 2. Probios® was slightly decreased with the addition of the lincomycin, whereas, the Great Lakes and the Fermented Products cultures were not affected by the addition of lincomycin to the cultures.

The effect of storage time on the stability of Probios® is shown in Table 3. Probios® had an initial count of 6.06 log CFU per gram of feed and by the end of the 12-week period the number of live organisms had decreased by 15%. The greatest reduction (10%) in viability occurred the first week.

The effect of the environments on the stability of Probios® is shown in Tables 4 and 5. These data suggest that by maintaining the cultures in refrigeration stability is prolonged. At room temperature the cultures were more stable than those maintained in the warm environment. The cultures stored in the nursery had the shortest shelf-life.

The stability of Lact-A-Bac® stored in complete feed in the environments are shown in Table 6. The cultures maintained in refrigeration has greater stability than those stored at room temperature or in the nursery. Cultures maintained in the nursery dropped substantially from a 8.6 to 4.6 log CFU within a week. This suggests that the majority (47%) of reduction in viability is occurring the first week after the culture is placed into the complete feed. By week seven there were no appreciable levels of lactobacillus detected.

The stability of lactobacillus products supplied by Fermented Products tended to have shorter shelf life than the other two products (Table 7). Refrigeration prolonged the stability of the Fermented Products culture. Cultures maintained at room temperature had slightly better stability than those maintained in the nursery. At room temperature and in the nursery substantial reduction in viable counts was observed the first week after mixing the Fermented Products culture in the feed as also observed with the Great Lakes Biochemical product. By week five cultures maintained at room temperature and in the nursery had no appreciable viability.

These data suggest that refrigeration will prolong (P<.05) stability of all three commercially available products. The stability of each product stored at room temperature was greater than those stored in the nursery (P<.05). Medication (lincomycin at 100 grams per ton of feed) had a minor affect on product viability. The product viability was reduced (P<.05) over the 12-week storage period and the majority of reduction viability was influenced most greatly the first week of storage. Therefore, it can be concluded that the stability of viable lactobacillus products is influenced by the storage environment (heat and humidity), length of storage and type of medication in the feed. If a viable culture is necessary for a growth response in pigs, it is necessary to use a product that will give adequate stability.

Table 1. Antibiotic Sensitivity

	Colony type	
Antibiotic	White	Ivory
Ampicillin	+	+
Cephalothin	-	+
Chloromycetin	+	+
Erythromycin	+	+
Furazolidone	+	+
Gentamycin	+	+
Kanamycin	+	+
Lincomycin	-	-
Methicillin	-	-
Neomycin	ND	+
Nitrofurazone	+	+
Penicilllin	+	+
Vetisuld	-	-
Streptomycin	+	+
Tetracycline	+	+
Triple Sulfa	-	-
Trimethoprim/sulfamethoxazole	+	+
Nitrofurantoin	ND	+

+ = sensitive
- = resistant
ND = not determined

Table 2. Medication Effect on Log CFU per g of Feed

Product	Lincomycin –	Lincomycin +[a]	
Pioneer	5.51	5.36	P<.10
Great Lakes	5.49	5.42	N.S.[b]
Fermented Products	3.94	3.69	N.S.

[a]100 g/ton of feed
[b]Nonsignificant

Table 3. Effect of Storage Time on Probios® Stability

Week	Log CFU/g of feed
0	6.06[a]
1	5.45[b]
3	5.45[c]
5	5.26[c]
7	5.22[cd]
12	5.16[d]

[a,b,c,d]Different superscript are significant (P<.05)

Table 4. Effect of Environment on Stability of Probios®

Environment	Log CFU/g of feed
Refrigerated	5.81[a]
Room temperature, sealed	5.43[b]
Room temperature, open	5.67[c]
Nursery, sealed	4.98[d]
Nursery, open	5.29[e]

[a,b,c,d,e]Different superscripts are significant (P<.05).

Table 5. Stability of Probios® Stored in Complete Feed in Several Environments[a]

Environment[b]	Time, weeks[c] 0	1	3	5	7	12
	--- Log CFU/g of feed ---					
Refrigerated	6.03	5.52	5.73	5.84	5.84	5.88
Room temp., sealed	6.02	5.52	5.64	5.35	5.20	4.83
Room temp, open	6.09	5.60	5.71	5.61	5.56	5.48
Nursery, sealed	5.09	5.40	4.88	4.49	4.49	4.52
Nursery, open	6.07	5.21	5.30	5.04	5.02	5.09

[a]Probios® - Pioneer Hi-Bred International
[b]Environment effect (P<.01)
[c]Time effect (P<.05)

Table 6. Stability of Lact-A-Bac® Stored in Complete Feed in Several Environents

Environment[b]	Time, weeks[c] 0	1	3	5	7	12
Refrigerated	8.59	8.17	6.73	6.62	6.08	7.11
Room temp, sealed	8.61	5.78	4.91	4.22	4.13	3.19
Room temp, open	8.62	6.35	5.01	4.74	4.22	3.98
Nursery, sealed	8.58	4.23	3.33	3.37	nil[d]	nil
Nursery, open	8.63	4.94	3.63	3.16	nil	nill

[a]Lact-A-Bac® - Great Lakes Biochemical Inc.
[b]Environment effect (P<.05)
[c]Time effect (P<.05)
[d]Too few to count

Table 7. Stability of Lactobacillus Stored in Complete Feed in Several Environents

Environment	Time, weeks[c] 0	1	3	5	7	12
Refrigerated	5.05	5.03	4.95	4.61	4.73	4.15
Room temp, sealed	5.06	4.09	2.19	nil[d]	nil	nil
Room temp, open	5.03	3.93	3.38	nil	nil	nil
Nursery, sealed	4.10	3.13	1.92	nil	nil	nil
Nursery, open	5.07	2.63	1.72	nil	nil	nil

[a]Lactobacillus from Fermented Products, Inc.
[b]Environment effect (P<.05)
[c]Time effect (P<.05)
[d]Too few to count