Enumeration and Identification of Lactobacilli in Feed Supplements Marketed as Sources of *Lactobacillus Acidophilus*

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Story in Brief

In recent years much interest has been demonstrated in using products containing *Lactobacillus acidophilus* as feed supplements to improve animal health. This interest has been partially due to the possibility that use of antibiotics in animal feeds may be banned. Fifteen of these feed supplements marketed by eight different manufacturers under various trade names were analyzed for numbers and types of lactobacilli. There was a wide variation with regard to the numbers of lactobacilli contained in the products. Very few of the products contained the organism, *Lactobacillus acidophilus* although they did contain other species of lactobacilli. Three of the products contained no detectable lactobacilli, and many of the others contained such low numbers of lactobacilli that it is doubtful that they would be beneficial when added to the feed for animals.

Introduction

In recent years much emphasis has been given to the possibility of using *Lactobacillus acidophilus* or certain other intestinal lactobacilli as dietary adjuncts for humans and animals to help control intestinal infections. A good deal of this interest in regard to livestock has been based on the possible banning of antibiotics as routine feed supplements.

There are many feed supplements on the market today which are being sold as sources of lactobacilli and other microorganisms reported to be beneficial to the intestinal well being of the animals. These preparations are indicated to be useful in preventing the occurrence of scours as well as improving growth and performance of livestock. Most of the products are marketed as dry materials designed to be mixed with the desired animal ration for usage. If the lactobacilli contained in such products are to establish and/or grow in the intestinal tract of the animal and thus help prevent the occurrence of intestinal infection, they should be viable. While there is no conclusive evidence available regarding the numbers of such lactobacilli required to be beneficial to the animal, it is generally accepted that a relatively high number of the organisms should be fed to the animal. The idea or basis for using organisms such as *L. acidophilus* as a dietary adjunct to help prevent the occurrence of intestinal infections is a good one; however, unless the appropriate species of lactobacillus is used in sufficient numbers, beneficial effects may not be realized.

Due to our interest in the use of *L. acidophilus* as a dietary adjunct, not only for humans but for animals, we obtained a number of commercially available products being marketed as feed supplements to be analyzed for the numbers and types of lactobacilli.

Materials and Methods

The products were prepared and analyzed for numbers of facultative and bile-resistant lactobacilli according to procedures published by Gilliland and Speck (1977). The numbers of bile-resistant lactobacilli are important since it is necessary that the lactobacilli be resistant to bile in order to grow in the intestinal tract. The bacteriological media used for the enumeration were lactobacillus selection (LBS) agar (prepared according to formulation of BBL, Division of Becton, Dickinson and Co., Cockeysville, MD) and lactobacillus selection agar plus 0.2 percent oxgall (LBSO) agar. The desired dilutions of each sample were plated with the appropriate agar, and the plates were incubated in a carbon dioxide atmosphere for 5 days before counting the colonies. For most of the samples which exhibited colonies of lactobacilli, two colonies from each media per sample were isolated for identification. The isolated lactobacilli were identified using methods described by Gilliland and Speck (1977). In addition to the test reported by them, each isolate was also tested for the presence of catalase and for the ability to grow at 15°C. Characteristics for the lactobacilli presented in the 8th Edition of *Bergey's Manual of Determinative Bacteriology* were used as bases for identification of the isolates. The identification involved 20 to 22 characteristics of each isolate.

Results and Discussion

There was a wide range in the numbers of lactobacilli identified in the 15 products tested. The populations ranged from <100/g up to 1.8×10^7/g (Table 1). Three of the products contained no detectable lactobacilli. A fourth one contained no detectable bile-resistant lactobacilli. There were only two products which contained more than one million viable lactobacilli per gram. Of these two products only one contained more than one million bile-resistant lactobacilli per gram.

If directions supplied by the manufacturers of the products were followed in feeding them, the animal would receive very few lactobacilli, based on the number of viable lactobacilli detected in this study. Of the eight products from which lactobacilli were isolated for identification, only one (Product No. 5) contained *L. acidophilus*. The

Table 1. Enumeration and identification of lactobacilli in feed supplements sold as sources of *Lactobacillus acidophilus*

	Count/g		Predominating species isolated	
Product[a]	LBS AGAR	LBSO AGAR	LBS AGAR	LBSO AGAR
1	7.8×10^5	3.0×10^5	*L. brevis*	*L. brevis*
2	2.0×10^5	2.0×10^5	—	—
3	4.0×10^3	3.7×10^3	*L. plantarum*	*L. plantarum*
4	1.1×10^7	7.5×10^6	*L. brevis*	*L. brevis*
5	7.3×10^3	2.1×10^4	*L. acidophilus*	*L. acidophilus*
6	1.8×10^7	2.5×10^4	*L. lactis*	*L. brevis*
7	4.0×10^2	<100	—	—
8	1.6×10^4	1.6×10^4	*L. brevis*	*L. brevis*
9	<100	<100	—	—
10	3.5×10^4	3.0×10^4	*L. brevis*	*L. brevis*
11	6.9×10^4	1.0×10^5	*L. brevis*	*L. brevis; L. casei*
12	7.0×10^2	9.0×10^2	—	—
13	<100	<100	—	—
14	<100	<100	—	—
15	1.2×10^3	1.6×10^3		

[a]All products were dry; each was given a code number prior to analyses.

number of lactobacilli in this product was quite low. The species of lactobacillus most often encountered in other products was *L. brevis*. While some of the manufacturers indicated more than one species of lactobacillus to be present in their products, *L. brevis* was not mentioned as one that was present in any of them. The species most often mentioned as being present in the animal feed supplements were *L. acidophilus* and *L. plantarum*.

Based on past research, the use of *L. acidophilus* and/or other intestinal lactobacilli appears to be a feasible means for helping control intestinal infections in livestock. However, this author is not aware of any studies involving the use of *L. brevis* or *L. plantarum* in this manner. If the organisms used as dietary adjuncts are to grow in the intestine and thus help prevent the occurrence of intestinal infections, they must first be viable in a product fed to the animal, and they must be present in sufficient numbers so that they may establish and/or manifest their action in the intestinal tract. While the minimum number required has not been established, it is generally accepted that a relatively high number must be ingested in order to have a beneficial effect. The numbers of lactobacilli detected in the products in the present study would most likely not be adequate to be beneficial.

There have been a number of studies reported in which feed supplements such as those reported in this study were tested for their effectiveness in controlling intestinal infections in various species of livestock. Results from these studies have been variable. Some have indicated positive results, others negative results. More often than not, these reports have not contained data showing the numbers of lactobacilli contained in the product, nor have they confirmed the identification of the organism(s) present. Another aspect not included has been the challenge of treated animals with pathogens that cause the intestinal infection. These are factors which must be considered in testing the effectiveness of feed supplements which are supposed to contain lactobacilli that are to function in the intestine.

Research must be conducted to develop procedures for preparing feed supplements which contain adequate numbers of the desirable lactobacilli in order to be useful in controlling intestinal infections in livestock. Factors which must be considered are: the viability of the organism during preparation and storage of the feed supplements, the numbers of lactobacilli that are necessary in the product and the most desirable species of the organism for such use. Most research to date would indicate that *L. acidophilus* would be the most desirable species; however, it is possible that some of the other species which can survive and grow in the intestinal tract might be equally as effective.

Literature Cited

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