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(54) Title: METHOD AND FORMULATION FOR REDUCING MICROBIAL POPULATIONS		
(57) Abstract <p>A formulation including one or more pure cultures of <i>Lactobacillus</i>, such as <i>L.reuteri</i>, <i>L.animalis</i> and <i>L.salivarius</i> and a sugar source, such as whey and a method of feeding animals which utilizes the formulation to be ingested by the animals with their normal food.</p>		

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METHOD AND FORMULATION FOR REDUCING MICROBIAL POPULATIONS

BACKGROUND OF THE INVENTIONField of the Invention

This invention relates generally to a method and
5 composition for reducing microbial populations, and more
particularly, to a composition containing Lactobacillus and
a carbohydrate for use in decreasing the numbers of
pathogens in the gastrointestinal tract which do not
utilize lactose, such as Salmonella.

10 Description of the Related Art

Lactobacillus reuteri, L. animalis, and L. salivarius
are naturally occurring microorganisms in the GI tract of
animals including domestic avian species (Sarraf, et al.,
1985, System Appl. Microbiol 6:86-89). This paper and all
15 other papers cited herein are hereby incorporated herein by
reference.

As disclosed in co-pending application Serial No.
07/268,361 filed September 19, 1988, the disclosure of
which is incorporated herein, Lactobacillus reuteri is a
20 symbiotic resident of the gastrointestinal (GI) tracts of
humans, swine and other animals. The neotype strain of L.
reuteri is DSM 20016 (ATCC No. 53609). This strain and
strain 1063 (ATCC No. 53608), discussed in the co-pending
application, are available to the public at the American
25 Type Culture Collection (Rockville, MD) having been
deposited therein April 17, 1987. The GI tract of animals
is a complex ecosystem harboring an estimated 300-500

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species of microorganisms, known collectively as the indigenous microbiota or microflora. Despite over 100 years of intensive research in the field of intestinal microbiology much remains to be learned about these
5 microorganisms, the complex interrelationships that exist between the different species, and the nature of the symbiotic relationships existent between the microbiota and their host.

Under certain conditions some members of the
10 indigenous microbiota can become opportunistic pathogens causing a variety of enteric diseases. More often, however, pathogens gain access to the GI tract as contaminants in food or water. Notable among the latter are a number of bacterial genera including Escherichia,
15 Salmonella, Shigella, Yersina, Vibrio, Campylobacter and Clostridium, as well as viruses (e.g., roto-, astro- and ciliciviruses) and intestinal parasites (e.g., Giardia and Entamoeba species). Acute and chronic enteric diseases caused by these and other microorganisms occur worldwide
20 causing considerable human misery and loss of economically important animals. Certain microbial activities have also been associated with production of mutagens within the GI tract.

It is also known that other members of the indigenous
25 microbiota exist in a symbiotic or synergistic relationship with their host contributing in many positive ways to the host's general health and well-being. It is well-known that germ-free animals are very susceptible to pathogens

and have poorly developed GI tracts. In return for the nutrient-rich and stable ecosystem provided for them, the indigenous microbiota can provide their hosts with an assortment of benefits including among others protection
5 against enteric pathogens (a process known as colonization resistance or competitive exclusion), stimulation of normal development and function of the GI mucosa, production of various vitamins and other nutrients, and re-metabolism of the host's abundant endogenous mucosal tissue.

10 It has been reported on numerous occasions that the enteric lactobacilli (i.e., bacteria belonging to the genus Lactobacillus which reside in the GI tract and which include a large number of nonpathogenic, non-toxic bacteria) play an important role in the health and well-
15 being of their human and animal hosts. Included in this role is their ability to regulate conditions in the GI tract so that the different populations of microorganisms in this system are kept in a healthy balanced state (Fuller, 1977, Br. Poultry Sci. 18:85-94). Certain
20 Lactobacillus species in fact are added to human and animal foodstuffs either to preserve them, enhance their flavors and/or exert other beneficial effects in the GI tract. Lactobacillus plantarum strains, for example, are grown commercially in large amounts and used as starter cultures
25 for the commercial preservation of a variety of human foods (meats, vegetables, and dairy products) and animal foods (silage). Lactobacillus acidophilus strains are grown commercially in large amounts to be added to human (e.g.,

milk) or animal (feedstuffs) foods as a means of introducing these bacteria into the GI tract where they can exert beneficial effects. Although these bacteria are likely to be already present in the GI tract their numbers may vary widely from individual to individual, and therefore beneficial effects of these bacteria may not be present in persons deficient in these bacteria. Reports on the beneficial effects resulting from the oral administration of live Lactobacillus cultures have increased in recent years with findings that dietary Lactobacillus therapy affords protection from colon cancer for human populations on western diets, reduces the incidence of experimentally induced large bowel tumors in rats, reduces the fecal concentration of bacterial enzymes known to catalyze the conversion of procarcinogens to proximal carcinogens in humans, and reduces the serum cholesterol levels in swine.

The metabolic endproducts of Lactobacillus metabolism, such as acetic acid, lactic acid and hydrogen peroxide, are well-known for their antimicrobial activities. They are believed to play a significant role in maintaining proper conditions within the GI tract. Some lactobacilli produce bacteriocins or bacteriocin-like proteins which also exhibit bacteriocidal activity toward other members of that species or closely related species. Reports have appeared concerning low molecular weight, antimicrobial substances produced by lactobacilli. With the exception of reuterin which is produced by Lactobacillus reuteri (copending

application) none of these low molecular weight substances has been identified and these reports have not been confirmed. In fact, some of these substances have proven to be none other than lactic acid, acetic acid or hydrogen
5 peroxide.

Several studies have been conducted to determine the effect of lactobacilli on the performance of domestic avian species. Some of these studies indicate that dosing broilers with L. acidophilus improved their growth
10 (Tortuero, 1973, Poultry Sci. 52:197-203, and Dilworth and Day, 1978, Poultry Sci. 57:1101). An increase in egg production as a result of addition of lactobacilli to laying hens feed has also been reported (Hargis and Creger, 1978, Poultry Sci. 57:1103).

15 Improvement of young turkeys' body weight and feed efficiency was obtained with a Lactobacillus product added to the feed (Francis et al., 1978, Poultry Sci. 57:1687-1689). Similar effects were reported (Potter et al., 1986, Poultry Sci. (Suppl. 1) 65:107) when L. acidophilus in
20 combination with varying protein levels was fed to turkeys up to 12 weeks of age, but no difference with the controls was observed at 16 weeks. Dosage of 10^7 colony forming units (CFU) and higher depressed chick growth (Watkins and Kratzer, 1982, Poultry Sci. 61:1565, and, 1984, Poultry
25 Sci. 63:1671-1673).

Other strains of lactobacilli did not stimulate broilers weight gain (Watkins and Kratzer, 1984), and did not stimulate egg production (Goodling et al., 1987,

Poultry Sci. 66:480-485). Damron et al. (1981, Poultry Sci. 60:1350-1351) did not find any beneficial effect of L. acidophilus and other lactobacilli cultures in turkey breeder hens.

5 Nurmi and Rantala (1973, Nature 241:210-211) demonstrated that the intestinal microflora present in some adult chickens (i.e. the cecal microflora) interferes with colonization by salmonellae of newly hatched chicks. The application of this concept, known as the Nurmi concept or
10 competitive exclusion, has been successfully tested in some laboratories and is also used commercially (Watkins and Miller, 1983, Poultry Sci. 62:1772-1779; Wierup et al., 1988, Poultry Sci. 67:1026-1033; Impey et al., 1982, J. Hyg. Camb. 89:749; Snoeyembos et al., 1978, Avian Dis.
15 22:273-287). There are many problems associated with this method, in particular, a lack of adequate selective isolation and characterization techniques to study and consistently obtain cecal flora preparations (Bailey, 1988, Poultry Sci. 67:928-932; Mead and Impey, 1986, J.
20 Bacteriol. Symp. Suppl. 675-755; Soerjadi et al., 1981, Avian Dis. 25:1027-1033.

Mannose and lactose were shown to significantly reduce Salmonella typhimurium adherence to the ceca of chicks (Oyofa et al., 1989, Avian Dis. 33:531-534). The
25 inhibitory effect of these sugars was believed to take place by blocking the receptor sites on the gut epithelium and on the microorganism pili. It has been shown that providing dietary lactose together with cecal flora

contents to broiler chickens reduced the occurrence of Salmonella (Corrier et al., 1990, Avian Dis. (in press); Hinton et al., 1990, Avian Dis. (in press); Oyofu et al., 1989).

5 Historically, Lactobacillus administration (i.e., inclusion of viable cells in the feed) to animals has not yielded consistent benefits. There are many reasons for this including, for example, using Lactobacillus species or strains unadapted to or unsuitable for the animal being
10 treated, or using conditions which do not produce a colonization of the Lactobacillus within the GI tract.

The object of this invention is to provide a food or feed additive formulation and method comprising isolated and identified pure cultures of Lactobacillus reuteri
15 and/or other Lactobacillus species together with a sugar source such as lactose, using whey as a source for this sugar.

Lactobacillus reuteri was previously found to be recoverable from the stools of piglets after the piglets
20 had been fed large quantities of the cells (co-pending application). It was hypothesized in the co-pending application that such cells could be used, possibly in conjunction with glycerol, as a growth and health aid for these animals. Evidence has been obtained (and presented
25 below) that L.reuteri may be administered to poultry as a means of competitively excluding salmonellae.

It is an object of this invention to provide a formulation that may be used as a food or food additive

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mixture that utilizes pure cultures of one or more of certain Lactobacillus species in addition to a sugar source.

It is a further object of the invention to provide a
5 formulation that results in rapid weight gain for growing animals.

It is a further object of the invention to provide a formulation that decreases the number of pathogenic microorganisms in the gastrointestinal tract, with the
10 purpose of adding any sugar for at least the purpose of being a source of carbohydrate for the metabolism of the Lactobacillus but not utilized by the animal or the unwanted microorganism(s).

Other objects and advantages will be more fully
15 apparent from the following disclosure and appended claims.

SUMMARY OF THE INVENTION

The invention is for a formulated product that may be used as an animal feed additive and that includes isolated and identified pure culture(s) of naturally occurring
20 gastrointestinal microorganisms, for example, Lactobacillus reuteri, L. animalis, and/or L. salivarius. The formulated product also includes a sugar source. The invention also includes a method of feeding the formulation to animals. Preferably the sugar source is whey, when
25 animals which do not metabolize lactose such as chickens are used, because whey contains the sugar lactose and is an easily obtainable and voluminous waste product.

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but not metabolizable by the animals in the group.

By the term "group" is meant animals of a particular species or group of species which share in common a tendency to have a similar gastrointestinal Lactobacillus flora and a similar inability to metabolize a sugar which is metabolizable by the Lactobacillus flora. As discussed below, the formulation discussed in detail herein has been devised for poultry but is adaptable to other animals, and includes a source of lactose which is not metabolizable by poultry.

In the preferred embodiment, the formulation comprises live, pure cultures of at least one of Lactobacillus reuteri, L. animalis and L. salivarius, and a sugar source.

The preferred sugar source is whey, because it is inexpensive and easily available, and because it contains lactose, a good source of carbon and energy for growth of the added microorganisms. An additional advantage of using a lactose source for feeding poultry or other birds is that birds do not utilize this sugar, and it is therefore readily available for the added microorganisms. Preferably, powdered whey is utilized as the lactose source to minimize shipping costs and spoilage prior to formulation of the additive.

The preferred method of formulating the additive is as follows. L.reuteri, L.animalis and L.salivarius are grown individually in a variety of appropriate media used for lactobacilli. Lactose or maltose are the preferred sources

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of energy so that the cells are capable of rapid metabolism of the carbohydrates which may be present in the formulation or in the animals' food. The cells used for the preparation of the additive may be freshly harvested, 5 frozen, lyophilized or suspended in oil or a specifically formulated diluent such as an aqueous solution. Commercially available whey powder or whey concentrate is used to formulate the additive. Although the cells and whey may be fed separately, they are preferably mixed 10 together with or without other ingredients (e.g. corn, soybean meal, wheat, etc.). The mixture may be of a variety of microbe and whey mixtures, for example a solid and a solid (e.g. fine powder with granulated whey, etc.), a liquid and a solid (cell suspension and whey), a solid 15 and a liquid (lyophilized cells and a liquid whey concentrate) or a liquid and a liquid (liquid cell suspension and a liquid whey concentrate). The additive final presentation of the mixture could be as a powder, granules, or pellets or liquid.

20 Animals may be fed the additive in a variety of ways: for example, (1) the additive may be combined with dry feed during feed milling or when the feed is delivered to the animals; (2) the additive may be sprinkled on the food as a powder; or (3) the additive may be mixed in the drinking 25 water. Preferably, to minimize labor, the additive is mixed with dry feed.

The features and advantages of the present invention will be more clearly understood by reference to the

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following examples, which are not to be considered as limiting the invention.

EXAMPLES

Example 1. Growth of Turkey Poults to be Fed Additive
5 Experimentally

One day old Nicholas turkey tom poults are used in this study. The poults are not toe clipped, desnooded or wing clipped, nor are they given any vaccinations.

10 The turkeys are placed in animal rooms at the Dearstyne Poultry Research Center, Department of Poultry Science at NCSU's Agricultural Research Service (NCARS). The animal rooms have controlled ambient temperature, day length and thermostatically controlled Petersime brooding batteries (Petersime Incubator Co., Petersime, OH).

15 A normal turkey starter diet, for example as shown in Table 1 with and without whey powder, is used throughout the trial. The amount of whey in the diet allows for a final 2.2% lactose. The trial is twenty days in duration, covering the period from day of hatch to day 12. The
20 turkeys are weighed on Day 0 (at hatch), Day 5, Day 12, Day 15 and Day 20.

Table 1.

TURKEY STARTER DIET			
5	INGREDIENT	CONTROL (lbs/1000)	PLUS WHEY (lbs/1000)
	Soy	490	487
	Corn	405	373
	Whey (73% lactose)	---	30
10	Poultry Fat	44.4	49.6
	Ethoxyquin	0.12	0.12
	Dicalcium phosphate	35.5	35.5
	Limestone	14.5	14.5
	Sodium Chloride	3.7	3.7
15	Trace minerals	1.0	1.0
	Vitamin mix,	1.0	1.0
	Choline Cl (60%)	2.0	2.0
	L-Lys.HCl	0.1	0.1
	DL-Met	2.8	2.8
20		<u>1000.0</u>	<u>1000.0</u>

Example 2. Growth and Quantitation of Bacterial Cultures

L.reuteri 11284, known to colonize the chicken GI
25 tract, and L.reuteri T1, which is a strain isolated from
turkeys, are the strains which are used. The
Lactobacillus strains are grown in LCM medium utilizing
lactose or maltose for 24 h at 37°C, harvested by
centrifugation, and washed twice with fresh basal medium as
30 previously described (Axelsson et al., 1989, Microbial

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Ecol. Health Dis. 2:131-136; Chung et al., 1989, Microbial
Ecol. Health Dis. 2:137-144). These cells are mixed into
the animal feed at a level of approximately 10^5 CFU g^{-1} of
feed. This inoculum level has been shown to effectively
5 enhance the population level of this microorganism in the
chicken ceca (Casas et al., 1990, in preparation). The
number of L. reuteri in the feed and in the ceca are
monitored as previously described (Chung et al., 1989,
Microbiol. 56: 943-948). Appropriate dilutions are plated
10 onto LBS agar and incubated anaerobically (Gas-Pak jars) at
37°C for 48 h. Plates containing about 50 to 200 CFU are
overlaid with glycerol agar seeded with L. plantarum
indicator cells, reincubated anaerobically for 24 h, and
colonies showing growth inhibition zones counted as
15 reuterin-producing L.reuteri cells.

S. senftenberg, isolated from turkeys, resistant to
novobiocin and nalidixic acid, was obtained from Evillmar
Poultry Co. (Evillmar, MN). Inocula for infectious
challenge are prepared from cultures grown in BHI Broth
20 (Difco Laboratories, Inc., Detroit, MI) and incubated at
37°C for 24 hours. The cultures are diluted appropriately
in sterile 50 mM phosphate buffer, pH 7.0, to obtain
challenge inocula containing 10^6 CFU per ml. Enumeration
of these Salmonella is carried out by plating appropriate
25 dilutions on Rembach Salmonella medium (Rembach, 1990,
Appl. Environ. Microbiol. 56:301-303).

Caecal content samples for microbiological enumeration
are prepared from sacrificed birds. Caeca are carefully

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removed from the birds and the open end of each is clipped. The exterior of the caecum is alcohol sterilized before transferring its contents to a stomacher bag for mixing and further dilution.

5 Example 3. Treatment of Poults

Turkey poults of Example 1 are subjected to the following eight treatments with two pens of 15 birds per pen being in each group:

Salmonella senftenberg infected group

- 10
1. Control, no whey, no L.reuteri
 2. No whey, L.reuteri
 3. Whey, no L.reuteri
 4. Whey, L.reuteri

L.reuteri, when administered is mixed into the feed.

15 The inoculated feed is changed every two days to guarantee the presence of viable L. reuteri in the feed. Whey is added to the feed, before milling, for a final 5% lactose concentration. Alternatively, L. reuteri and concentrated or dehydrated whey are formed into tablets , or added

20 together in any product in which both components in a liquid or solid form have been previously combined, and the combination added to the feed of the animals.

S. senftenberg (10^6 CFU per ml) is crop fed by the means of an animal feeding stainless steel needle attached

25 to a hypodermic syringe on day 5 after hatch.

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Example 4. Results of Addition of Lactobacillus, Whey and Salmonella

Salmonella senftenberg in feces and caecal contents of poult treated as in Example 3 is shown in Tables 2 and 3, respectively. The effect of L. reuteri and whey on the number of S. senftenberg in feces (droppings) becomes obvious at 72 h after Salmonella challenge. The data indicate a synergistic effect when whey and L. reuteri are added together.

The presence of S. senftenberg in caecal contents is presented in Table 3. The results show that addition of L. reuteri and/or whey, but in particular, the combination of L. reuteri and whey, is effective in reducing the presence of S. senftenberg in the ceca of these animals. Thus, whereas 47% of the control ceca tested positive for S. senftenberg, none (0%) of the samples tested positive when fed L. reuteri and whey.

TABLE 2. Log₁₀ CFU of S. senftenberg per g feces 72 h post challenge.

REPLICA SAMPLES	TREATMENTS			
	CONTROL	<u>L.reuteri</u>	WHEY	WHEY AND <u>L.reuteri</u>
a.	> 10	6	3	4
b.	9	7	8	5
c.	> 10	7	6	< 3
d.	10	3	< 3	< 3

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TABLE 3. Percent of cecal samples testing positive for S. senftenberg and L. reuteri 7 days post challenge

	TREATMENTS	<u>L.reuteri</u> (%) ¹	<u>S.senftenberg</u> (%) ²
5	CONTROL	0	47
	<u>L.reuteri</u>	29	40
	WHEY	6	13
10	WHEY AND <u>L.reuteri</u>	82	0

¹ Positive samples had > 10⁷ CFU/g

² Positive samples had < 10³ CFU/g

15 Example 5. Growth of Cold Stressed Poults Fed with L. reuteri and Whey

Instead of exposing turkey poults to constant temperature rooms as in Example 1, the temperature in the pens of cold stressed birds is 90 degrees F for 1 hour, 20 then 85 degrees G for 2 hours in an on-off cycling for 48 hours after hatch. The temperature is then set back to normal brooding temperature for the remainder of the experiment, normal brooding temperature being 90 degrees F for the first seven days after hatch, 85 degrees F from day 25 7 to day 10, and 75 to 80 degrees after day 10.

Turkey poults are subjected to the following four treatments, with eight pens of eight birds per pen being in each treatment group:

1. Control, no whey, no L. reuteri
- 30 2. No whey, L. reuteri

3. Whey, no L. reuteri

4. Whey, L. reuteri

Example 6. Results of Growth of Cold Stressed Poults

Relative weights of poults treated as in Example 5, at 0, 5, 10, 15, and 20 days of age are shown in Table 4. The beneficial effect of whey becomes evident at day 5, while the effect of L. reuteri becomes obvious at days 15 and 20.

TABLE 4. Effect of L. reuteri and whey on body weight of turkey poults.

10

TREATMENTS	RELATIVE WEIGHT (PERCENT) AT				
	Day 0	Day 5	Day 10	Day 15	Day 20
15 No whey, no <u>L. reuteri</u>	100	100	100	100	100
No whey, plus <u>L. reuteri</u>	99	98	102	104	105
Plus whey, no <u>L. reuteri</u>	99	103	101	101	103
20 Plus whey, plus <u>L. reuteri</u>	99	103	103	100	105

For percentage conversion, control weights (no whey, no L. reuteri) at each weight day were made equal to 100%.

25

Example 7. Use of Lactobacillus salivarius and Lactobacillus animalis

L. salivarius subsp. salivarius ATCC type strain No. 11741 and L. animalis ATCC type strain No. 35046 are grown

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as in Example 2. Each strain is added individually to feed
as in Example 3. The feed is augmented with whey according
to Example 3. The feed is used to feed chickens and
turkeys to decrease undesirable microbial organisms and
5 improve poultry weight gain.

Example 8. Use of Multiple Lactobacillus Strains

Strains of the three Lactobacillus species discussed
in Examples 3 and 8 are each added individually, or as a
mixed inoculum to the whey-augmented feed according to
10 Example 3. The feed containing the three strains is used
to feed turkeys and chickens.

BEST MODE FOR CARRYING OUT THE INVENTION

The food or feed additive for animals, such as poultry
and mammals, including human beings, comprises one or more
15 pure cultures of a Lactobacillus species naturally
occurring in the gastrointestinal tract of that animal and
a source of a sugar that is metabolizable by the
Lactobacillus species but not to any great extent by the
animal. Preferably, the formulation comprises live, pure
20 cultures of at least one of Lactobacillus reuteri, L.
animalis and L. salivarius, and a sugar source. The
preferred sugar source is whey, particularly for poultry.
Whey is inexpensive and easily available, contains lactose,
a good source of carbon and energy for growth of the added
25 microorganisms, and is not utilized poultry or other birds
and therefore readily available for the added

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microorganisms. Preferably, powdered whey is utilized as the lactose source to minimize shipping costs and spoilage prior to formulation of the additive.

The preferred method of formulating the additive is as follows. L.reuteri, L.animalis and L.salivarius are grown individually in a variety of appropriate media used for lactobacill. The cells used for the preparation of the additive may be freshly harvested, frozen, lyophilized or suspended in oil or a specifically formulated diluent such as an aqueous solution. Commercially available whey powder or whey concentrate is used to formulate the additive. The cells and whey are preferably mixed together with or without other ingredients (e.g. corn, soybean meal, wheat, etc.). Animals are preferably fed the additive as a mixture with dry feed.

INDUSTRIAL APPLICABILITY

The invented formulation may be used as a food or food additive mixture for animals grown commercially, such as poultry. It provides a product that results in rapid weight gain for growing animals, and decreases the number of pathogenic microorganisms in the gastrointestinal tract, thereby resulting in increased productivity and decreased animal mortality.

While the invention has been described with reference to specific embodiments thereof, it will be appreciated that numerous variations, modifications, and embodiments

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are possible, and accordingly, all such variations, modifications and embodiments are to be regarded as being within the spirit and scope of the invention.

THE CLAIMSWhat Is Claimed Is:

1. A formulation for oral administration to an animal of a particular group, comprising:
 - 5 (a) a bacterial culture comprising at least one live pure culture of at least one Lactobacillus species which occurs naturally in a particular animal group; and
 - 10 (b) a source of sugar metabolizable by the Lactobacillus species in the bacterial culture but not metabolizable by the animals in the group.

2. A formulation for oral administration according to Claim 1, comprising a Lactobacillus culture selected from
15 the group consisting of L.reuteri, L.salivarius and L.animalis, and the animals are poultry.

3. A formulation for oral administration according to Claim 1, wherein the source of sugar comprises whey.

4. A formulation for oral administration according to
20 Claim 1, wherein the Lactobacillus culture is selected from the group consisting of L.reuteri, L.salivarius and L.animalis, and the source of sugar is whey.

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5. A formulation for oral administration according to Claim 4, further comprising glycerol.

6. A formulation for oral administration according to Claim 1, wherein pure cultures of L.reuteri, L.salivarius and L.animalis are used, and the sugar is lactose.

7. A formulation for oral administration according to Claim 6, wherein the source of lactose is whey.

8. A formulation for oral administration to animals, comprising:

- 10 (a) a bacterial culture consisting of at least one live, pure culture of Lactobacillus; and
(b) a source of sugar.

9. A formulation for oral administration according to Claim 8, comprising a Lactobacillus culture selected from the group consisting of L.reuteri, L.salivarius and L.animalis, and the animals are poultry.

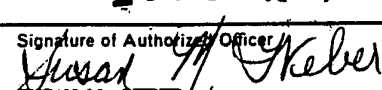
10. A formulation for oral administration according to Claim 8, wherein the source of sugar comprises whey.

11. A formulation for oral administration according to Claim 8, wherein the Lactobacillus culture is selected from the group consisting of L.reuteri, L.salivarius and L.animalis, and the source of sugar is whey.

12. A formulation for oral administration according to Claim 11, further comprising glycerol.
13. A formulation for oral administration according to Claim 8, wherein pure cultures of L.reuteri, L.salivarius and L.animalis are used, and the sugar is lactose.
14. A formulation for oral administration according to Claim 13, wherein the source of lactose is whey.
15. A method of decreasing numbers of undesirable microbes in an animal's gastrointestinal tract, comprising:
- 10 (a) obtaining at least one pure culture of Lactobacillus cells;
- (b) obtaining a source of sugar; and
- (c) administering the Lactobacillus cells and the sugar orally to the animal.
- 15 16. A method of decreasing numbers of undesirable microbes in gastrointestinal tracts of animals, comprising:
- (a) obtaining at least one pure culture of Lactobacillus cells;
- (b) obtaining a source of sugar metabolizable by the Lactobacillus cells and not to a significant extent by the animals or the undesirable microbes; and
- 20 (c) administering the Lactobacillus cells and the sugar orally to the animal.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/03796

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC (5): A01N 63/00; C12N 1/00, 1/20; A23L 1/00 U.S. Cl.: 424/93; 435/252.9, 853; 426/2		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S. Cl.	424/93; 435/252.9, 853; 426/2,41,43	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X Y	US, A, 3,984,575 (FARR) 05 OCTOBER 1976, See entire disclosure.	1-16
Y	US, A, 4,518,696 (GEHRMAN ET AL) 21 MAY 1985, See col.1, lines 5-52.	1-16
P, Y	US, A, 4,980,164 (MANFREDI ET AL) 25 DECEMBER 1990, See entire disclosure.	1-16
P, Y	US, A, 4,946,791 (MANFREDI ET AL) 07 AUGUST 1990, See entire disclosure.	1-16
Y	US, A, 4,591,499 (LINN ET AL) 27 MAY 1986, See entire disclosure.	1-16
Y	US, A, 4,689,226 (NURMI ET AL) 25 AUGUST 1987, See entire disclosure.	1-16
<p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
10 OCTOBER 1991	19 NOV 1991	
International Searching Authority	Signature of Authorized Officer	
ISA/US	 SUSAN M. WEBER	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US,A, 3,988,440 (Bogdanov) 26 October 1976, See entire disclosure.	1-16
Y	US,A, 4,314,995 (Hata et al) 09 February 1982, See abstract.	1-16
Y	JP,A, 62-130,680 (Calpis Shokuhin Kog) 03 December 1985. See abstract.	1-16

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers _____, because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

3. Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

The additional search fees were accompanied by applicant's protest.

No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Microbial Ecology in Health and Science, Volume 2, issued 1989, Chung et al, "In Vitro Studies on Reuterin Synthesis by <u>Lactobacillus reuteri</u> ", pages 137-144. See entire document.	1-16
Y	Dobrogosz et al., "Regulatory and Protective Role of the Normal Microflora", published - 1989, see pages 283-292.	1-16
T	Poultry Science, Volume 70, Supplement 1, issued, Parkhorst et al ^{Issue 1, 1991} "Lactobacillus reuteri and Dietary Whey Effect on Twenty Day Body Weights of Turkey Poults Subjected to Either Cold or Low Protein Stress", p 1-7, see entire disclosure.	1-16