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NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión
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SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

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(54) Title: IONOPHORE ANTIBIOTIC FORMULATIONS

(57) Abstract

An aqueous base suspension concentrate or use of ionophore antibiotic(s) capable of aqueous dilution (if desired) and capable (with or without such aqueous dilution) of being orally administered to an animal (e.g. by drenching) to prevent or treat conditions treatable by an ionophore antibiotic such as monensin (e.g. bloat, coccidiosis, et al), said concentrate comprising: (I) at least one ionophore antibiotic in (II) an aqueous system containing: (i) optionally a wetting and/or surfactant agent, (ii) optionally, an antifreeze agent or agents, in which the ionophore antibiotic or antibiotics is or are no more than sparingly soluble (preferably organic, e.g. a glycol), (iii) a suspension agent (optionally Xanthan gum), (iv) optionally an antifoam agent or system (optionally a Simethicone and particulate carrier system), (v) optionally a preservative, (vi) optionally a de-bittering agent, (vii) optionally a pH buffering system, and (viii) water, wherein (iii) is Xanthan gum or at least two of (i), (ii) and (iii) are present in (II).

not in any organic inclusion that has been added in as an antifreeze agent.

We have also found (irrespective of whether or not any antifreeze agent is present) that an ionophore antibiotic (such as monensin) can be stably suspended in aqueous suspension by Xanthan Gum that (i) holds the suspension stable when not subjected to shear but which shear thins, (ii) does not significantly age thicken, (iii) is capable of stably supporting a wide range of trace elements of biological significance (especially if in a chelated form) and (iv) is microbiologically stable.

The present invention therefore envisages an aqueous base suspension concentrate that stably supports an ionophore antibiotic, or mixtures thereof, at least in part with Xanthan Gum.

We have also found that as a wetting agent for enabling the mixing of an ionophore antibiotic (especially such as monensin) was much easier using an alkyl polyglycoside over other wetting agents.

The present invention therefore envisages an aqueous base suspension concentrate that stably supports an ionophore antibiotic, or mixtures thereof, where at least some of the antibiotic was mixed with water in the presence of alkyl polyglycoside.

It is believed that an aqueous based suspension concentrate in accordance with the present invention together with its diluted form and its use will provide the public with a useful choice.

In a first aspect the invention consists in **an aqueous base suspension concentrate of an ionophore antibiotic or ionophore antibiotics** capable of aqueous dilution (if desired) and capable (with or without such aqueous dilution) of being orally administered to an animal (eg; by drenching), said concentrate comprising

(I) at least one ionophore antibiotic (preferably in a crystalline and/or mycelial form) in (II) an aqueous system containing

(i) a wetting and/or surfactant agent

(ii) an antifreeze agent or agents in which the ionophore antibiotic or antibiotics is or are no more than sparingly soluble,

(iii) a suspension agent (preferably with rheological properties),

(iv) optionally an antifoam agent or system,

(v) optionally a preservative

(vi) optionally a de-bittering agent,

(vii) optionally a pH buffering system, and

(viii) water.

Preferably one or more (preferably all) of (II) (iv), (v), (vi) and (vii) are present.

In a second aspect the invention consists in **an aqueous base suspension concentrate of an ionophore antibiotic or ionophore antibiotics** capable of aqueous dilution (if desired) and capable (with or without such aqueous dilution) of being orally administered to an animal (eg; by drenching), said concentrate comprising

(I) at least one ionophore antibiotic (preferably in a crystalline and/or mycelial form) in (II) an aqueous system containing

(i) a wetting and/or surfactant agent

(ii) optionally an antifreeze agent or agents in which the ionophore antibiotic or antibiotics is or are no more than sparingly soluble,

(iii) a suspension agent (preferably with rheological properties),

(iv) as an antifoam agent or system, Simethicone and a particulate carrier therefor (preferably silicon dioxide)

(v) optionally a preservative

(vi) optionally a de-bittering agent,

(vii) optionally a pH buffering system, and

(viii) water.

Preferably one or more (preferably all) of (II) (ii), (v), (vi) and (vii) are present.

Preferably (i) is alkyl poyglycoside.

Preferably (ii) is a glycole.

Preferably (iii) is Xanthan Gum.

In a third aspect the invention consists in **an aqueous base suspension concentrate of an ionophore antibiotic or ionophore antibiotics** capable of aqueous dilution (if desired) and capable (with or without such aqueous dilution) of being orally administered to an animal (eg; by drenching), said concentrate comprising

(I) at least one ionophore antibiotic (preferably in a crystalline and/or mycelial

non-mycelial form) in an aqueous system which includes an organic antifreeze agent or agents but where most of the antibiotic is suspended in the aqueous system rather than dissolved in the organic antifreeze agent, the antibiotic being supported by a suspension agent.

Preferably said suspension agent is Xanthan Gum.

Preferably said aqueous fraction includes at least one or more of a wetting, buffering, preservative and antifoam and debittering agent.

In still a further aspect the present invention consists in **an aqueous base suspension concentrate of an ionophore antibiotic or ionophore antibiotics** (preferably sodium monensin) capable of aqueous dilution (if desired) and capable (with or without such aqueous dilution) of being orally administered to an animal (eg; by drenching), said concentrate comprising the at least one ionophore antibiotic (preferably in a crystalline non-mycelial form) in an aqueous system which includes an organic antifreeze agent or agents but where most of the antibiotic is suspended in the aqueous system rather than dissolved in the organic antifreeze agent, the antibiotic being supported by a suspension agent **wherein** said antibiotic has been mixed into at least some of the water in the presence of a wetting agent and subsequently has been supported as a suspension by the suspension agent.

In still a further aspect the invention consists in ~~a method of preparing an~~ aqueous base suspension concentrate of an ionophore antibiotic or ionophore antibiotics capable of aqueous dilution (if desired) and capable (with or without such aqueous dilution) of being orally administered to an animal (eg; by drenching), said concentrate comprising

(I) ~~(~~at least one ionophore antibiotic in ~~(II)~~ an aqueous system containing

(i) ~~a~~ wetting and/or surfactant agent

(ii) an antifreeze agent or agents in which the ionophore antibiotic or antibiotics is or are no more than sparingly soluble,

(iii) ~~(~~a suspension agent~~,)~~

(iv) optionally an antifoam agent or system,

(v) optionally a preservative,

MonoPotassium phosphate Dihydrate	0.04% w/v	Buffer
Dialkyl dimethyl ammonium bromide	.0064% w/v	Preservative
Sorbitol	3.5% w/v	Debittering agent
Xanthan Gum	0.4% w/v	Suspension agent
Alkyl Polyglycoside	0.5% w/v	Surfactant/Wetting agent
Simethicone	0.333% w/v	Anti-foam
Silicon Dioxide	0.167% w/v	Carrier for Simethicone
Water to 100%		

Previously was set out have options for the components of the concentrate and choices available including in respect of the antibiotic. The preferred form of the present invention however administers monensin and specifically sodium monensin in its crystalline form.

Crystalline monensin can be prepared in a number of different ways. Such a product is available from Eli Lilly & Co, Indianapolis, USA or can be prepared by way of the procedures set out, by way of example, in US Patent 4,213,966 (E.R. Squibb & Sons Inc).

For stability of the ionophore antibiotic an alkaline pH and preferably one about 8 - 8.2 is desired. The phosphate solvent system preferably buffers for this pH range.

The concentrates of the present invention has a stable shelf life of at least six months and can be decanted in metered amounts at anytime over its shelf life.

The components:

1. Antifreeze Agent (optional in some formulations)

Monoprolene glycol (MPG). It was found that it functioned adequately as an antifreeze.

Was useful to dissolve as a "premix" with DDAB (dialkyl dimethyl ammonium bromide - a preservative with some wetting agent characteristics) and xanthan gum (the preferred aqueous suspension agent for monensin).

Monensin was only very sparingly soluble in MPG without ultrasonic mixing and/or heating.

Other antifreeze agents can be utilised and it would be envisaged that in this

respect many glycerol derivatives or mixtures there of could equally be chosen. Care however must be exhibited since a gylcol (such as ethylene glycol) can be very toxic to monogastrics and cause kidney damage.

Since MPG works well and is safe and well known and used in the animal health industry, it is the antifreeze of choice.

2. The Suspension Agent.

The suspension of choice is xantham gum, (a hydrocolloid) which has the advantage of locking the suspension when not mobilised but soon as movement is required it shear thins. Unlike many other gums, it also does not age thicken.

Xanthan gum was found to be far more effective than guar gum and other such gums which tended to produce a solid lump in the bottom of the container after a period of time.

Xanthan gum was also very stable with respect to ions such as MGO, ZNO. Moreover xanthan gum is microbiologically stable.

3. The Ionophore Antibiotic

Monensin as sodium monensin was the preferred ionophore antibiotic.

4. The Buffering System

The buffers are of any effective compatible buffering system of a kind set forth.

5. The Preservative

The preservative DDAB was preferably added into the formulation early so as to make best use of its wetting effect in addition to its intended preservative effect. Its presence provides broad spectrum activity from the preservative point of view while at the same time providing some measure of antibloat activity due to its wetting effect.

6. The Wetting Agent

The preferred wetting agent was clearly alkyl polyglycoside. Its molecular structure was most compatible with monensin and enabled monensin to enter the aqueous phase easier than other agents tried. It again is a component to be added early into the preparative process.

7. The Antifoaming Agent

The preferred anti foaming agent is preferably gensil, an emulsion of simethicone and silicon dioxide.

39. A method of claim 37 or 38 wherein said administration is of sodium monensin at a daily rate per ruminant animal of from 0.4 milligrams per Kg body weight of animal to 2 milligrams per Kg body weight of animal.

40. A method of forming an aqueous base suspension concentrate of an ionophore antibiotic or ionophore antibiotics capable of aqueous dilution (if desired) and capable (with or without such aqueous dilution) of being orally administered to an animal (eg; by drenching), said concentrate comprising

(I) at least one ionophore antibiotic in (II) an aqueous system containing

(i) a wetting and/or surfactant agent

(ii) an antifreeze agent or agents in which the ionophore antibiotic or antibiotics is or are no more than sparingly soluble,

(iii) a suspension agent,

(iv) optionally an antifoam agent or system,

(v) optionally a preservative,

(vi) optionally a de-bittering agent,

(vii) optionally a pH buffering system, and

(viii) water,

which comprises

mixing the antibiotic to an aqueous system already containing at least some of the antifreeze agent(s), the optional preservative (if present and having a wetting effect), and the antifoaming agent or system, and

subsequently mixing in the balance of components (optionally with some measure of premixing).

41. A concentrate formed by the method of claim 40.

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United States Patent [19]

DeCampos et al.

[11] **Patent Number: 6,086,633**

[45] **Date of Patent: *Jul. 11, 2000**

[54] **METHOD FOR PRESERVING ANIMAL HIDES**

[75] Inventors: **Rogerio B DeCampos**, Sao Paulo, Brazil; **Martin K Kemmerling**, Clinton, Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **09/284,692**

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Primary Examiner—Alan Diamond
Attorney, Agent, or Firm—Frederick D. Hunter

[57] **ABSTRACT**

Compositions and methods for preserving untanned animal hides wherein the compositions comprise 50–95% (w/w) of a polyether antibiotic and 5–25% (w/w) of a surface acting agent. In these compositions, greater than 90% of the polyether antibiotic has a particle size of less than 25 microns, and the surface-acting agent is present in an amount that is effective to disperse the polyether antibiotic uniformly in an aqueous suspension.

27 Claims, No Drawings

METHOD FOR PRESERVING ANIMAL HIDES

This application is a 371 of PCT/US96/16840 filed Oct. 17, 1996.

BACKGROUND OF THE INVENTION

This invention relates to compositions and methods for preserving animal hides, in particular, protecting them against degradation resulting from microbiological growth.

Animal hides received by tanneries in excellent condition can be used for valuable end products and sold at a premium as compared to lesser quality hides. However, prior to arriving at a tannery, freshly stripped hides are typically washed and stored; during this period, such hides are susceptible to decomposition by microorganisms. Microbial growth on the hide surface and within the hide and blood vessels may cause irreparable damage resulting in lower quality hides having reduced value. Damaged hides may display holes or darkened areas, reduced hide strength, and altered stretching characteristics. Hair slippage, color, and integrity, all measures of hide quality, may also be adversely affected by microbial growth.

A variety of agents have been used for preserving hide quality but numerous detrimental effects are associated with their use. The oldest and most widely used preservation process is treatment of the hides with sodium chloride. Salt reduces the water content of the hides creating an adverse environment for microbial growth. Concentrated salt solutions also have a certain bactericidal activity. Typically, 0.3–0.6 kg sodium chloride per kg of wet hide is added to control microbial growth. However, the addition of such agents as sodium chloride also provokes hide shrinkage that may be considered a loss. Moreover, the sodium chloride is corrosive to equipment and its use results in significant, negative environmental impact on discharge or additional expense when removed prior to discharge.

The rot-preventing effect of salt can be enhanced by the addition of chemical biocidal or bactericidal agents, thereby reducing the amount of salt required. Such additives include, for example, naphthalene, p-chloro-m-creosol, sodium silicofluoride, ortanotin compounds, chlorinated phenols, pyridine derivatives, quaternary ammonium compounds, zinc salts, monochloroacetic acid, and many others. However, all of these additives require the concomitant use of a relatively high quantity of salt.

Likewise, antibiotics, such as tetracyclines, streptomycin, and penicillin, have also been proposed for use in preserving hides by inclusion in a hot brine solution. This method also contemplates optionally injecting the antibiotic into the animal prior to slaughter followed by immersion in the antibiotic-containing hot brine solution.

Numerous attempts to develop salt-free preservation processes have been largely unsuccessful. Such attempts include drying, solvent dehydration, bactericidal treatment, irradiation, and cooling. These processes have been generally unreliable for a variety of reasons, e.g., damage to the hides, toxicological and environmental safety problems, limited effectiveness, and/or economically unviable.

The present invention addresses the need to preserve hides without damage and eliminate the adverse economic and environmental impacts associated with the use of other agents and processes.

SUMMARY OF THE INVENTION

The present invention provides a novel composition useful for preserving untanned animal hides comprising:

(a) 50–95% (w/w) of a polyether antibiotic wherein greater than 90% of the particles are less than 25 microns in size, and

(b) 5–25% (w/w) of a surface-acting agent effective to uniformly disperse the polyether antibiotic in an aqueous suspension.

Accordingly, the present invention also provides a method for preserving untanned animal hides comprising contacting the hides with an aqueous suspension containing at least 0.1 g/l of the above described composition, preferably 1–10 g/l of the composition, and especially 1, 5, or 10 g/l.

It is preferred that the composition and method comprise a polyether antibiotic having a median particle size of about 7–10 microns.

Moreover, it is preferred that the composition comprise 70–90% (w/w) of a polyether antibiotic, especially 80%. Likewise, it is preferred that the composition comprise 5–20% (w/w) of a surface-acting agent, more preferably 10–20%, especially about 12–17%.

Thus, an especially preferred method comprises, for example, contacting animal hides with 5 g/l of a composition that contains 80% polyether antibiotic and 12–17% surface-acting agent, that is, 4 g/l polyether antibiotic and 0.6–0.85 g/l surface-acting agent in the final hide-preserving suspension. Such a method is particularly applicable for use when the hides are to be sprayed with the suspension. Suspensions containing 1 g/l of the composition (0.8 g/l polyether antibiotic; 0.12–0.17 g/l surface-acting agent) are also particularly suited for use when the hides are to be immersed in the suspension.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel composition comprising bacteriostatic or bactericidal concentrations of polyether antibiotics having a reduced particle size and amounts of surface-acting agents effective to uniformly disperse the polyether antibiotic in an aqueous suspension, such composition being useful for preserving untanned animal hides.

The composition of the current invention is desirable for use by hide removal and processing plants for a variety of reasons. The polyether antibiotic active agents are very stable compounds. They do not easily degrade over time or under high temperature, remaining active for many days under typical hide storage conditions. Polyether antibiotics are effective at low concentrations in hide processing, thereby minimizing the quantities being delivered to waste treatment systems and keeping the cost of such treatment low. Moreover, aerobic waste treatment systems are not affected by the antibiotic activity, presumably since the polyether antibiotics are partially or completely degraded under aerobic conditions. These characteristics provide the opportunity to process animal hides free of salt.

Experiments conducted on freshly removed hides, using compositions of the current invention containing sodium monensin crystals that had been air milled to a reduced particle size and mixed with sodium lauryl sulfate, demonstrated that the antimicrobial activity of such compositions was adequate to preserve hide quality for a time sufficient to allow processing and transport to a tanning facility. In this way the detrimental effects associated with sodium chloride use were avoided. Moreover, the effectiveness of such compositions on control of odor, hair loosening (slippage), hide color, and soluble nitrogen concentration also demonstrated their usefulness for hide processing. Significantly,

compositions of the current invention are better able to preserve hides than currently available commercial chemical antimicrobial agents when compared to the industry's reported experience with, and the advertised claims of, such products.

It will be recognized that various polyether antibiotics will likewise be suitable for use in the current invention. Examples of suitable polyether antibiotics include monensin, lonomycin, ionomycin, nigericin, grisorixin, dianemycin, lenoremycin, antibiotic X206, alborixin, septamycin, antibiotic A204, compound 47224, mutalomycin, salinomycin, lasalocid, isolasalocid A, lysocellin, tetronasin, echeromycin, antibiotic X14766A, antibiotic A23187, antibiotic A32887, compound 51532, K41, semduramycin, narasin, maduramycin, and laidlomycin. The use of monensin in the current invention is preferred, and the use of sodium monensin is especially preferred. The skilled artisan will recognize that other polyether antibiotics are also suitable for use in the current invention.

The current composition may be applied to the hides by direct application including dipping or immersing the hides in a suspension of the current composition, sprinkling or spraying the suspended composition onto the hides, or any other method of application whereby the composition comes into contact with the hides. Concentrations of composition of at least 0.1 g/l in the treatment suspension are effective. Given the designated range of active polyether antibiotic of 50–95% (w/w) that may be included in the composition, the concentration of polyether antibiotic in a hide-preserving suspension using 0.1 g/l of composition would be 0.05–0.095 g/l. The use of 1–10 g/l of composition is preferred (0.5–9.5 g/l of polyether antibiotic). The use of 1, 5, or 10 g/l of composition (0.5–0.95 g/l, 2.5–4.75 g/l, or 5–9.5 g/l polyether antibiotic, respectively) is further preferred.

Since the polyether antibiotics are only slightly soluble in the aqueous environment typically utilized for animal hide processing, in order to achieve effectiveness, the polyether antibiotic must be uniformly dispersed in an aqueous suspension. According to the current invention, the polyether antibiotic may dispersed by the addition of a broad range of surface-acting agents, including, for example, various fatty acids, linear alkyl sulfonates, and alkyl benzene sulfonates. Specific surface-acting agents which are suitable for use in the current invention include, for example, sodium lauryl sulfate, ammonium lauryl sulfate, triethanolamine lauryl sulfate, sodium caprylic acid, sodium cholic acid, sodium lauryl ether sulfate, ammonium lauryl ether sulfate, lauryl dimethyl amine oxide, sodium N-lauroylsarcosin, benzalkonium chloride, cetylpyridinium chloride, various polyoxyethylene ethers, and various polyoxyethylenesorbitans. Compositions having 10–20% (w/w) surface-acting agent are preferred; those having about 12–17% surface-acting agent are more preferred. Sodium lauryl sulfate is a preferred surface-acting agent for the current invention.

Uniform dispersion is also affected by particle size. Polyether antibiotics having a particle size distribution wherein at least 90% of the particles are less than 25 microns may be used in the current invention. A distribution of particles having a median size of about 7–10 microns is preferred. For purposes of the current invention, all measurements of particle size were done according to laser diffraction technology and reported as diameters.

Of course, the effectiveness of suspensions created by using the current compositions is dependent upon the interaction of particle size and the concentration of surface-acting agent. As particle size is reduced, the requirement for a surface-acting agent also decreases. It is a matter of skill in the art to determine effective ratios of polyether antibiotic to surface-acting agent for any given particle size. Relative amounts of polyether antibiotic and surface-acting agents expressed as percents in this disclosure are calculated on a weight basis (w/w).

The fermentation of organisms responsible for production of the various polyether antibiotics and subsequent isolation steps typically result in polyether antibiotic crystals of greater than 20 microns in size (generally 20–50 microns), and having a purity of 80–98%. Thus, the isolated crystals may contain 2–20% undefined inert matter. This crystalline material may be processed according to a number of known procedures to reduce the particle size to the required specifications. Air milling is particularly suited to micronize the particles to the extent necessary.

The processed material is then mixed directly with the surface-acting agent by any means suitable thereby generating the current compositions. Mechanical mixing or air mixing, for example, may be used in the current invention.

For ease in use, the composition of the current invention can be formulated into a package that can be added to an aqueous phase to generate a suspension containing the desired concentrations of polyether antibiotic and surface-acting agent. For example, it is particularly contemplated that a 100 g package containing 80 g (80% (w/w)) crystalline monensin and approximately 12–17 g (12–17% (w/w)) sodium lauryl sulfate be prepared. A 1000 g package containing 800 g crystalline monensin and 120–170 g sodium lauryl sulfate is also contemplated. For particular convenience, the packaging material may be made of water soluble materials such that the package may be added to the aqueous diluent without having to separately prepare the composition thereby avoiding contact with the ingredients.

The present invention is contemplated as being useful in a wide variety of situations, including, but not limited to, the preserving of untanned hides from the following species: bovine (including Bos taurus and Bos indicus animals), porcine, ovine, caprine, equine, fish (including eel), avian (including ostrich, emu, and other fowl), reptiles (including alligators, snakes and lizards), fur animals (including rabbit, raccoon, beaver, muskrat and other rodents) and other exotic species such as yak, vicunas, llamas, camels, or any other type of animal hide used in the manufacture of leather or production of pelts for use in taxidermy or decoration. Thus, any animal hide that can be tanned for leather, taxidermy, decoration or other uses can be processed in accordance with the present invention.

The following examples are intended for illustrative purposes and are not intended and should not be construed as limiting the invention in any way.

EXAMPLE 1

Composition Preparation

Sodium monensin crystals (92.7% pure) were air milled. The air mill device used was typical in that the crystals were introduced into a high speed air stream that conveyed the crystals with high turbulence into an impact and classifying chamber. The crystals were reduced by interparticular impact and attrition. The reduced particles are expelled and the oversize particles are retained for further milling. After milling the monensin had a particle size distribution as follows:

Particle Size (microns)	% of Sample less than size indicated
1.09	5
1.68	10
3.45	25
7.20	50
22.34	99
28.46	95
52.50	100

172.6 Kg of the milled crystals were mixed in a blender with 27.4 kg of sodium lauryl sulfate (industrial grade) resulting in a composition containing 80% sodium monensin and 13.7% sodium lauryl sulfate.

The composition s o prepared was then used for a number of experimental trials described in the following examples.

EXAMPLE 2

Impact on Hide Quality

Compositions prepared as in Example 1 were suspended in water with stirring at varying concentrations. Freshly harvested hides were then immersed in tanks containing the various suspensions (Tank). Other fresh hides were sprayed with approximately 3 liters/hide of the various suspensions (Spray). The monensin treated hides were then stacked and stored at ambient temperature.

Preservation of monensin-treated hides was evaluated at various times by reference to parameters typically used to subjectively assess hide quality, such as hair slippage, odor, and color. These indicators were compared to (1) those for hides receiving no antimicrobial treatment, but which were stored at refrigerated temperatures, and (2) untreated hides that were stored under the same conditions as the treated hides.

The results are presented in Table 1.

TABLE 1

Storage Time (hrs)	Quality Parameter	Refrig. Temp.	Ambient Temp.	Treatment: Tank 0.1 g/l	Tank 0.5 g/l	Tank 1 g/l	Spray 1 g/l	Spray 5 g/l	Spray 10 g/l
24	Hair Slip	+	+	+	+	+	+	+	+
	Odor	+	±	±	±	+	+	+	+
	Color	+	+	+	+	+	+	+	+
48	Hair Slip	+	−	+	+	+	+	+	+
	Odor	+	±	±	±	+	+	+	+
	Color	+	−	±	±	±	+	+	+
72	Hair Slip	+	−	+	−	−	±	+	+
	Odor	+	−	±	±	±	±	+	+
	Color	+	−	−	−	−	±	+	+
96	Hair Slip	+	−	−	−	−	−	±	±
	Odor	+	−	−	−	−	±	±	+
	Color	+	−	−	−	−	−	±	+
192	Hair Slip	+	−	−	−	−	−	−	±
	Odor	+	−	−	−	−	−	−	±
	Color	+	−	−	−	−	−	−	±

+ No degradation detected
± Initial stages of degradation detected
− Degradation detected

EXAMPLE 3

Impact on Hide Quality

Freshly harvested hides were either immersed in tanks containing suspensions of the hide-preserving compositions (Tank) or treated with sprays of the suspensions (Spray) as in Example 2 at concentrations indicated in Table 2. The monensin-treated hides were again stacked and stored at ambient temperature.

Hide quality parameters for the treated hides were compared to those for refrigerated, untreated hides. The results are presented in Table 2.

TABLE 2

Storage Time	Test	Refrig. Control	Treatment: Tank 0.1 g/l	Tank 5 g/l	Spray 5 g/l
24 hrs	Hair Slip	+	+	+	+
	Odor	+	+	+	+
	Color	+	+	+	+
48 hrs	Hair Slip	+	+	+	+
	Odor	+	+	+	+
	Color	+	+	+	+
72 hrs	Hair Slip	+	+	+	+
	Odor	+	+	+	+
	Color	+	+	+	+
96 hrs	Hair Slip	+	+	+	+
	Odor	+	+	+	+
	Color	+	+	+	+
192 hrs	Hair Slip	+	±	±	±
	Odor	+	±	±	±
	Color	+	±	±	±

+ No degradation detected
± Initial stages of degradation detected
− Deterioration detected

EXAMPLE 4

Production-Scale Hide Treatment

Three separate large-scale plant trials (approximately 3.2 metric tons of hide) were conducted in two different leather processing plants. Fresh hides were immersed in suspension (prepared as in Examples 2 and 3) of high-preserving composition at a concentration of 1 g/l and subjected to mild agitation. Hide degradation was measured using the same subjective tests as Examples 2 and 3 for a 120 hours. The results are shown in Table 3.

TABLE 3

Trial #	Storage Temp	Hours of Storage 48	72	96	120
1	17–29 C.	+	ND	ND	ND
2	12–22 C.	+	+	+	ND
3	13–23 C.	+	+	+	Hair slip detected

+ No degradation detected
± Initial stages of degradation detected
– Degradation detected
ND Test Not Done

EXAMPLE 5

Effect on Soluble Nitrogen Concentration

Degradation of hides is related to protein metabolism. Thus, increases in the measure of soluble nitrogen compared to the total nitrogen present is an indication of degradation. Table 4 illustrates the effect of the current composition upon protein degradation in hides stored at ambient temperature for up to 96 hours compared to untreated controls stored at either refrigerated or ambient temperature. Hides were either immersed (Tank) or sprayed (Spray) with suspensions of hide-preserving composition at the concentrations indicated in Table 4. Soluble nitrogen concentrations were measured at 24 hour intervals.

TABLE 4

Treatment	Composition (g/l)	Time of Storage (hrs) 0	24	48	72	96
Refrig. Untreated	—	0.6%	ND	ND	ND	ND
Ambient Untreated	—	0.6%	1.1%	2.9%	4.8%	6.9%
Tank	0.1	0.6%	1.0%	ND	3.2%	ND
Tank	0.5	0.6%	1.2%	1.7%	3.1%	ND
Tank	1.0	0.6%	1.2%	ND	1.7%	3.1%
Spray	1.0	0.6%	ND	ND	3.3%	ND
Spray	5.0	0.6%	ND	1%	1%	1.9%
Spray	10.0	0.6%	ND	ND	ND	1.0%

ND Test Not Done

We claim:

1. A composition for preserving untanned animal hides comprising:

(a) 50–95% (w/w) of a polyether antibiotic wherein greater than 90% of the antibiotic has a particle size of less than 25 microns, and

(b) 5–25% (w/w) of a surface-acting agent effective to uniformly disperse the polyether antibiotic in an aqueous suspension.

2. The composition of claim 1 comprising 70–90% of the polyether antibiotic.

3. The composition of claim 1 comprising about 80% of the polyether antibiotic.

4. The composition of claim 1 or 3 wherein the polyether antibiotic is sodium monensin.

5. The composition of claim 1 or 3 comprising 10–20% (w/w) of the surface-acting agent.

6. The composition of claim 1 or 3 comprising about 12–17% (w/w) of the surface-acting agent.

7. The composition of claim 1 or 3 wherein the surface-acting agent is sodium lauryl sulfate.

8. The composition of claim 4 comprising about 12–17% (w/w) sodium lauryl sulfate as the surface-acting agent.

9. The composition of claim 1 or 3 wherein the polyether antibiotic has a median particle size of about 7–10 microns.

10. The composition of claim 8 wherein the polyether antibiotic has a median particle size of about 7–10 microns.

11. A method for preserving untanned animal hides comprising contacting the hides with an aqueous suspension containing at least 0.1 g/L of a composition comprising

(a) 50–95% (w/w) of a polyether antibiotic wherein greater than 90% of the antibiotic has a particle size of less than 25 microns, and

(b) 5–25% (w/w) of a surface-acting agent effective to disperse the polyether antibiotic uniformly in an aqueous suspension.

12. A method of claim 11 wherein the polyether antibiotic comprises about 80% of the composition.

13. A method of claim 11 wherein the suspension contains 1–10 g/L of the composition.

14. A method of claim 12 wherein the suspension contains 1–10 g/L of the composition.

15. A method of claim 11 wherein the polyether antibiotic is monensin.

16. The method of claim 13 wherein the polyether antibiotic has a median particle size of about 7–10 microns.

17. A method of claim 15 wherein the polyether antibiotic has a median particle size of about 7–10 microns.

18. A method of claim 15 wherein the surface-acting agent is sodium lauryl sulfate and comprises 12–17% of the composition.

19. A method of claim 15 wherein the aqueous suspension contains 1–10 g/L of the composition.

20. The method of claim 13 wherein the suspension contains 1 g/L, 5 g/L, or 10 g/L of the composition.

21. The method of claim 14 wherein the suspension contains 1 g/L, 5 g/L, or 10 g/L of the composition.

22. The method of claim 14 wherein the polyether antibiotic has a median particle size of about 7–10 microns.

23. The method of claim 18 wherein the polyether antibiotic has a median particle size of about 7–10 microns.

24. A kit comprising:

(a) about 80% (w/w) of a polyether antibiotic wherein greater than 90% of the antibiotic has a particle size of less than 25 microns; and

(b) 12–17% (v/v) of a surface-acting agent, wherein the polyether antibiotic and the surface-acting agent are packaged in a container, which is optionally water soluble.

25. A kit of claim 24 wherein the polyether antibiotic is sodium monensin having a median particle size of about 7–10 microns and the surface-acting agent is sodium lauryl sulfate.

26. A kit of claim 25 weighing a total of 1000 grams.

27. A kit of claim 25 weighing a total of 100 grams.

* * * * *

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ANTIBIOTICS MONENSIN AND A204 FOR IMPROVING RUMINANT FEED EFFICIENCY

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U.S. Cl. 424—115 12 Claims

ABSTRACT OF THE DISCLOSURE

Antibiotics of the group A204, X537A, dianemycin, monensin, nigericin, and X206, the deshydroxymethyl derivatives of monensin and nigericin, and their physiologically-acceptable salts and esters, improve the digestive efficiency of certain herbivorous animals. Oral administration of the antibiotics to ruminant animals having a developed rumen function, and to animals which ferment fibrous vegetable matter in the cecum, changes the digestive fermentation to produce more propionates relative to the production of acetates, thereby improving feed utilization.

CROSS-REFERENCE

This application is a continuation-in-part of my copending application Ser. No. 220,304, filed Jan. 24, 1972.

BACKGROUND OF THE INVENTION

For many years, the animal science industry has tried to increase the efficiency of feed utilization by animals. The ruminant animals are of particular economic importance, and so, necessarily, is the efficiency of the utilization of ruminants' feed.

In the course of investigating the efficiency of feed use, the mechanism by which ruminants digest and degrade the components of their feed to form molecules which can be metabolically utilized has been intensively studied. The mechanism of carbohydrate utilization is now well known. Microorganisms in the rumen of the animal ferment the carbohydrate to produce monosaccharides, and then degrade the monosaccharides to pyruvate compounds.

It is to be understood that the rumen produces 2-carbon, 3-carbon, and 4-carbon compounds in the form of acids and salts and other derivatives of the acids. Since it is impossible to identify precisely what form the various compounds take, those compounds are referred to in the art respectively as acetates, propionates, and butyrates.

Pyruvate is then metabilized by microbiological processes to either acetates or propionates. Two acetate compounds may be combined thereafter, still in the rumen, to form a butyrate. Leng, "Formation and Production of Volatile Fatty Acids in the Rumen," *Physiology of Digestion and Metabolism in the Ruminant* (Phillipson *et al.* ed.), Oriel Press, pages 408–10.

The animal can utilize butyrates, propionates, and acetates with differing degrees of efficiency. Utilization of those compounds, which are collectively known as volatile fatty acids (VFA), occurs after absorption from the gut of the animal. Butyrates are utilized most efficiently, and acetates the least efficiently. However, the relative efficiency of use of butyrates is negated by the inefficiency

of the manufacture of butyrates, which must be made from acetates in the rumen.

One of the major inefficiencies in the rumen is in the manufacture of acetates. Since they are made by the degradation of pyruvates, each molecule of an acetate which is produced is accompanied by a molecule of methane. Most of the methane produced is lost through eructation. Since a butyrate is made from two acetate molecules, each molecule of the relatively efficiently used butyrates results in the loss to the animal of two molecules of methane, with all of the associated energy.

Thus, the efficiency of carbohydrate utilization can be increased by treatments which cause the animal to produce propionates rather than acetates from the carbohydrates. Further, the efficiency of feed use can be effectively monitored by observing the production and concentration of propionate compounds in the rumen. If the animal is making more propionates, it will be found to be using its feed more efficiently.

The relative efficiency of utilization of the VFA's is discussed by McCullough, *Feedstuffs*, June 19, 1971, page 19; Eskeland *et al.*, *J. Anim. Sci. 33*, 282 (1971); and Church *et al.*, *Digestive Physiology and Nutrition of Ruminants*, vol. 2 (1971), pages 622 and 625.

It has been well established that the efficiency of feed utilization by a ruminant animal can be readily determined by chemical analysis of the fermentation occurring in the rumen. For example, Marco *et al.*, U.S. Pat. 3,293,038 taught the use of alkylated phenols as feed additives for improved feed efficiency. They illustrated an *in vitro* rumen fermentation test, and *in vivo* animal feeding studies, which were evaluated by chemical analysis of the rumen contents for acetates and propionates.

O'Connor *et al.*, *J. Anim. Sci. 30*, 812–18 (1970), reported the results of *in vitro* rumen fermentation tests on a large number of compounds. German Pat. 2,059,407 reported the use of a hemiacetal of chloral and starch as a feed additive which inhibits the formation of methane and produces higher than normal levels of propionic and butyric acids.

Marco *et al.*, U.S. Pat. 3,522,353 taught the use of halogenated acyclic carboxylic acids as feed additives. It was there shown that the compounds produced *in vitro* increases in propionate production, and also increased feed efficiency in animals fed those compounds. To a similar effect is Erwin *et al.*, U.S. Pat. 3,564,098.

The condition called ketosis is a manifestation of faulty VFA balance, which amounts to a clinical illness. Ruminant animals maintained on a diet which naturally degrades to a high proportion of acetates and low proportion of propionates are likely to suffer from ketosis. Dairy animals are particularly prone to the condition. Under stress, such as the onset of high lactation, insufficient propionates are available. As a result, more acetates are used leading to a high concentration of ketones in the body and especially in the bloodstream. A treatment for ketosis is to feed propionic acid, a precursor of propionic acid, or glucose which tends to metabolize to propionates. Clearly, if the rumen could be caused to produce more propionates than normal from the diet, ketosis incidence could be reduced.

SUMMARY

I have now discovered a novel method of increasing the efficiency of feed utilization by ruminant animals

dered diluent, such as a sugar, starch, or purified crystalline cellulose in order to increase its volume for convenience in filling capsules.

Tablets of the antibiotics of my method are made by conventional pharmaceutical processes. Manufacture of tablets is a well-known and highly advanced art. In addition to the active ingredient, a tablet usually contains a base, disintegrator, an absorbent, a binder, and a lubricant. Typical bases include lactose, fine icing sugar, sodium chloride, starch and mannitol. Starch is also a good disintegrator as is alginic acid. Surface-active agents such as sodium lauryl sulfate and dioctyl sodium sulphosuccinate are also sometimes used. Commonly-used absorbents again include starch and lactose while magnesium carbonate is also useful for oily substances. Frequently-used binders are gelatin, gums, starch, dextrin and various cellulose derivatives. Among the commonly-used lubricants are magnesium stearate, talc, paraffin wax, various metallic soaps, and polyethylene glycol.

My method can also be practiced by the administration of the antibiotic compound as a slow-pay-out bolus. Such boluses are made as tablets are made except that a means to delay the dissolution of the antibiotic is provided. Boluses are made to release for lengthy periods. The slow dissolution is assisted by choosing a highly water-insoluble form of the antibiotic. A substance such as iron filings is added to raise the density of the bolus and keep it static on the bottom of the rumen.

Dissolution of the antibiotic is delayed by use of a matrix of insoluble materials in which the drug is embedded. For example, substances such as vegetable waxes, purified mineral waxes, and water-insoluble polymeric materials are useful.

Drenches of my antibiotics are prepared most easily by choosing a water-soluble form of the antibiotic. If an insoluble form is desired for some reason, a suspension may be made. Alternatively, a drench may be formulated as a solution in a physiologically-acceptable solvent such as polyethylene glycol.

Suspensions of insoluble forms of my antibiotics can be prepared in nonsolvents such as vegetable oils such as peanut, corn, or sesame oil; in a glycol such as propylene glycol or a polyethylene glycol; or, in water, depending on the form of the antibiotic chosen.

Suitable physiologically-acceptable adjuvants are necessary in order to keep the antibiotic suspended. The adjuvants can be chosen from among the thickeners, such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many classes of surfactants serve to suspend antibiotics. For example, lecithin, alkylphenol polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters are useful for making suspensions in liquid nonsolvents.

In addition many substances which affect the hydrophilicity, density, and surface tension of the liquid can assist in making suspensions in individual cases. For example, silicone anti-foams, glycols, sorbitol, and sugars can be useful suspending agents.

The suspendable antibiotic may be offered to the grower as a suspension, or as a dry mixture of the antibiotic and adjuvants to be diluted before use.

My antibiotics may also be administered in the drinking water of the ruminants. Incorporation into drinking water is performed by adding a water-soluble or water-suspendable form of the desired antibiotic to the water in the proper amount. Formulation of the antibiotic for addition to drinking water follows the same principles as formulation of drenches.

The most practical way to treat animals with the antibiotic compounds of my method is by the formulation of the compound into the feed supply. Any type of feed may be medicated with the antibiotic compounds, including common dry feeds, liquid feeds, and pelleted feeds.

The methods of formulating drugs into animal feeds are well known. It is usual to make a concentrated drug premix as a raw material for medicated feeds. For example, typical drug premixes may contain from about one to about 400 grams of drug per pound of premix. The wide range results from the wide range of concentration of drug which may be desired in the final feed. Premixes may be either liquid or solid.

The formulation of ruminant feeds containing the proper amounts of my antibiotic compounds for useful treatment is well understood. It is necessary only to calculate the amount of compound which it is desired to administer to each animal, to take into account the amount of feed per day which the animal eats and the concentration of antibiotic compound in the premix to be used, and calculate the proper concentration of antibiotic compound, or of premix, in the feed.

All of the methods of formulating, mixing, and pelleting feeds which are normally used in the ruminant feed art are entirely appropriate for manufacturing feeds containing the antibiotic compounds of my method.

It is not my intention to limit the scope of my invention to any particular formulations or methods of administration. My invention is a method of increasing the efficiency of feed utilization by ruminant animals by the oral administration of certain antibiotics. However the administration may be accomplished, it remains my method.

It is usual to treat economic animals, including ruminants, with a variety of growth promoters, disease-preventives, and disease treatments throughout their lives. Such drugs are often used in combination. My method may be practiced in combination with other treatments.

As has been shown, oral administration of the antibiotics effective in my method beneficially alters the production of propionates relative to the production of acetates in the rumen. The same treatment also benefits monogastric animals which ferment fibrous vegetable matter in the cecum. The monogastric animals here referred to are those which consume fibrous vegetable food and digest at least part of it by microbiological fermentation in the cecum. The cecal fermentation follows a chemical pathway similar to rumen fermentation.

Horses, swine, and rabbits are exemplary animals which digest a part of their food by cecal fermentation. The overall feed utilization of such animals is improved by the oral administration of the antibiotics effective in my method by means of a beneficial change in the propionate/acetate ratio. Horses and rabbits are exemplary of animals in which cecal fermentation is a major part of the total digestive process, and in which my antibiotics are accordingly particularly beneficial.

I claim:

1. A method of increasing the efficiency of feed utilization by ruminant animals having a developed rumen function which comprises the oral administration to such animals of a propionate-increasing amount of an antibiotic chosen from the group consisting of monensin, the deshydroxymethyl derivative of monensin, and the physiologically-acceptable esters and salts thereof.

2. A method of Claim 1 in which the compound administered is chosen from the group consisting of monensin and its physiologically-acceptable esters and salts.

3. A method of Claim 2 wherein the compound is administered at a rate of from about 0.05 mg./kg./day to about 2.5 mg./kg./day.

4. A method of Claim 3 in which the ruminant animals are cattle.

5. A method of increasing the efficiency of feed utilization by ruminant animals having a developed rumen function which comprises the oral administration to such animals of a propionate-increasing amount of an antibiotic chosen from the group consisting of A204, monensin, the deshydroxymethyl derivative of monensin, and the physiologically-acceptable esters and salts thereof.

6. A method of Claim 5 wherein the ruminant animals are cattle.