(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 251 748 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Drawings
Drawing(s) replaced or added

(48) Corrigendum issued on:
28.05.2008 Bulletin 2008/22

(45) Date of publication and mention of the grant of the patent:
09.01.2008 Bulletin 2008/02

(21) Application number: **01904801.6**

(22) Date of filing: **25.01.2001**

(51) Int Cl.:
A23L 1/0532 *(2006.01)* ***A23K 1/14*** *(2006.01)*

(86) International application number:
PCT/US2001/000649

(87) International publication number:
WO 2001/054515 (02.08.2001 Gazette 2001/31)

(54) REDUCING E. COLI CONTENT OF BEEF

REDUZIERUNG DES E. COLI-GEHALTES IN RINDFLEISCH

REDUCTION DU CONTENU EN E. COLI DANS LE BOEUF

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(30) Priority: **31.01.2000 US 494349**

(43) Date of publication of application:
30.10.2002 Bulletin 2002/44

(73) Proprietor: **TEXAS TECH UNIVERSITY**
Lubbock, TX 79409-2007 (US)

(72) Inventors:
- **ALLEN, Vivien Gore**
  Lubbock, TX 79407-2252 (US)
- **POND, Kevin, R.**
  Wolfforth, TX 79382 (US)

(74) Representative: **Burford, Anthony Frederick**
Beck Greener
Fulwood House
12 Fulwood Place
London WC1V 6HR (GB)

(56) References cited:
BE-A- 626 884 **US-A- 5 229 118**

- **DATABASE WPI Section Ch, Week 199211 Derwent Publications Ltd., London, GB; Class B04, AN 1992-084363 XP002224499 & JP 04 026628 A (NIPPON N U S KK), 29 January 1992 (1992-01-29)**
- **BURGESS J G ET AL: "Microbial antagonism: a neglected avenue of natural products research" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 70, no. 1-3, 30 April 1999 (1999-04-30), pages 27-32, XP004173381 ISSN: 0168-1656**
- **ALLEN ET AL.: 'Effects of seaweed treatment of tall fescue on grazing steers' PROCEEDINGS OF AMERICAN FORAGE AND GRASSLANDS COUNCIL vol. 6, 1997, GEORGETOWN, pages 168 - 172, XP002939125**
- **VACCA ET AL.: 'The antibacterial activity of an extract obtained from ascophyllum nodosum' JOURNAL AMER. PHARMACEUTICAL ASSOCN. vol. XLIII, no. 1, January 1954, pages 24 - 26, XP002939137**
- **HOBBS D.: 'Seaweed 'solution' for Livestock stress' THE NEW FARM May 1994 - June 1994, pages 26 - 28, XP002939138**
- **WOODWARD L.: 'The seaweed solution' VISTAS, TEXAS TECH. RESEARCH vol. 8, no. 1, 1999, pages 20 - 25, XP002939126**

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

• **ANONYMOUS: "E coli O157 in Dairy cattle: Questions and Answers" INTERNET CITATION, [Online] Retrieved from the Internet: URL:http://ohioline.osu.edu/vme-fact/0016. html> [retrieved on 2004-11-01]**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

[0001] This invention relates to a method of producing beef of reduced pathogenic *E. coli* content by the use of seaweed supplement.

[0002] Beef is typically obtained from cattle (beef cattle or dairy cattle) that have grazed in pastures and have fed in feedlots. About 18 to 24 months after birth, the animals are slaughtered and primal cuts are obtained.

[0003] The bacterium *E. coli* is a natural inhabitant of the gastrointestinal (GI) tract of most wann-blooded animals, including man and cattle. Over 200 serotypes or strains of *E. coli* have been identified, and most are non-pathogenic. In 1971, however, *E. coli* was first recognized as a cause of food borne illness in the United States when contaminated imported cheese caused diarrheal disease in about 400 individuals. Evidence that certain strains ofE. *coli* were associated with human disease grew, and now five classes of "enterovirulent" E. *coli* are recognized as food borne pathogens causing diseases ranging from traveler's diarrhea to the potentially deadly hemorrhagic colitis/hemolytic uremic syndrome. Red meat (including beef) and poultry have been implicated as prominent sources of enterovirulent E. *coli.* One strain of enterovirulent E. *coli* is O157:H7. Presence of this specific strain is considered as an adulterant by the USDA.

[0004] At slaughter of cattle, the carcases are inspected and graded and tested for *Eschericia coli* O157:H7 (*E. coli* 0157:H7) content on a random basis. Ground beef is also randomly tested specifically for *E. coli* O157: H7. Meat found with *E. coli* O1 57:H7 is not suitable for human consumption and is destroyed. Meat found negative for *E. coli* O157:H7 and with an acceptable level of *E. coli* content or which were not tested for *E. coli* content is usually vacuum packaged to preserve freshness, and the packaged meat is sent to supermarkets or other meat distribution businesses where the vacuum packaging is removed and the meat is repackaged or displayed in a case for sale, or cuts are converted into ground meat or cut into smaller cuts which are repackaged or displayed in a case for sale.

[0005] We turn now to approaches for reducing the pathogen content of beef. One approach in the battle against food borne pathogens is to reduce the occurrence of these pathogens at the farm. Many food borne pathogens (including E. *coli* O157:H7) are inhabitants of the GI tract and feces of cattle. During slaughter and processing, fecal contaminants from the hide, hair and hooves can be transferred to the carcass, and in current processing schemes there is no foolproof means of eliminating these pathogens from raw meat products. Since the origin of these pathogens tends to be the GI tract, it is thought that by manipulating animal feed, the environment of the gut can be altered to enhance the growth of healthy non-pathogenic bacteria in the gut, and/or minimize or eliminate the presence of pathogens; this approach has not been entirely successful.

[0006] It is conceived herein that a heightened immune response could have a positive impact on microflora in the gut and that administration of seaweed supplement as a result of causing heightened immune response or otherwise (e.g., direct toxicity to *E. coli* microbes) will result in production of beef with reduced pathogenic *E. coli* content thereby improving the safety of beef and beef products.

[0007] In one embodiment of the invention herein the seaweed supplement is directly feed to cattle during the feedlot finishing period of the life cycle of beef production.

[0008] In another embodiment of the invention herein the seaweed supplement is incorporated on or into forage during cattle grazing.

[0009] The invention also provides a method of obtaining beef of reduced E. *coli* O157:H7 content comprising the steps of: (a) directly feeding seaweed supplement to cattle during the feedlot finishing period of the life cycle of beef production; (b) slaughtering the cattle and obtaining meat; and (c) analyzing for E. *coli* O157:H7 content in samples of the meat; thereby to obtain and demonstrate the presence of beef of reduced E. *coli* O157:H7 content compared to if seaweed supplement were not fed.

[0010] The invention further provides a method of obtaining beef of reduced E. *coli* 0157:H7 content comprising the steps of: (a) grazing cattle on forage on or into which seaweed supplement has been incorporated; (b) slaughtering the cattle and obtaining meat; and (c) analyzing for pathogenic *E. coli* content in samples of the meat; thereby to obtain and demonstrate the presence of beef of reduced pathogenic E. *coli* content compared to if seaweed supplement were not fed.

[0011] The invention herein is directed to the use of seaweed in the manufacture of a supplement for use during the feedlot finishing period of the life cycle of beef production and/or incorporated on or into forage during cattle grazing to reduce pathogenic E. *coli* content of meat obtained on slaughter of the cattle.

[0012] The term "meat" is used herein to include primal cuts as well as smaller cuts, including ground meat.

In the Drawings:

[0013]

Fig. 1 is a graphic presentation of effect of feeding of seaweed extract on fecal *E. coli* content and shows results of Example I;

Fig. 2 is a graphic presentation of effect of feeding of seaweed extract on hide *E. coli* content and shows results of Example I;

Fig. 3 is a graphic presentation of effect of feeding of seaweed extract on fecal *E. coli* 0157:H7 content and shows results of Example I; and

Fig. 4 is a graphic presentation of effect of feeding of seaweed extract on hide *E. coli* 0157:H7 content and shows results of Example I.

[0014] In both of the embodiments herein, the cattle can be beef cattle or dairy cattle that are not used for milk production or breeding purposes or can be cull cows from breeding herds and milking herds.

[0015] We turn now to the seaweed supplements for the two embodiments.

[0016] The seaweed supplement is, for example, seaweed extract or seaweed meal.

[0017] The seaweed from which the seaweed supplement is obtained can be from any of the various seaweed plant classifications, preferably those that have been utilized in agriculture and include seaweeds from the plant orders *Laminariaceae, Fucaceae* and *Gigartinaceae.* Genus groups include *Ascophyllum, Laminaria, Durvillea, Macrocystis, Chondrus* and *Ecklonia.* The seaweed for the preferred seaweed supplement herein is from the genus *Ascophyllum* which belongs to the order *Fucaceae* and is *Ascophyllum nodosum. Ascophyllum nodosum* is a brown seaweed which grows along the North Atlantic shorelines of Canada, the United States, and Europe.

[0018] We turn now to seaweed supplement which is seaweed extract.

[0019] Seaweed extract is water soluble and can be obtained by alkaline hydrolysis extraction. A preferred seaweed extract is obtained by alkaline hydrolysis extraction from *Ascophyllum nodosum;* commercial products of this kind are available from Acadian Seaplants Limited of Nova Scotia Canada, and are sold under the tradenames Acadian Soluble Seaweed Extract Powder (powder form), Acadian Liquid Seaweed Concentrate (liquid form), Tasco™-Ex (powder form) and Tasco™-Forage (powder form). Acadian Soluble Seaweed Extract Powder, Tasco™-Ex and Tasco™-Forage have the same composition. Acadian Soluble Seaweed Extract Powder is made up of brownish-black crystals, has a seaweed-like odor, is 100% soluble in water and has a pH of 10-10.5 in water and typical analysis shows by weight 6.5% maximum moisture, 45-55% organic matter, 45-55% ash (minerals), 1.0 - 2.0% total nitrogen (N), 2.0 - 4.0% available phosphoric acid (P_2O_5), 1 8.0 - 22.0% soluble potash (K_2O), 1.0 - 2.0% sulfur (S), 0.2 - 0.5% magnesium, 0.1 - 0.2% calcium, 3.0 - 5.0% sodium, 75-150 ppm boron, 75 - 250 ppm iron, 8 - 12 ppm manganese, 1 - 10 ppm copper, 25 - 75 ppm zinc; alginic acid, mannitol, and laminarin carbohydrates; cytokinin, auxin and gibberellin growth promoters; and the following average grams of amino acid per 100 grams of protein: alanine, 3.81; arginine, 0.22; aspartic acid, 5.44; cystine, trace; glutamic acid, 7.69; glycine, 3.16; histidine, 0.42; isoleucine, 1.94; levcine, 4.84; lysine, 1.33; methonine, 1.39; phenylalanine, 2.82; proline, 4.42; serine, 0.14; threonine, 1.27; tyrosine, 1.80, and valine, 3.46.

[0020] We turn now to seaweed supplement which is seaweed meal or flour.

[0021] The seaweed meal or flour can be obtained by dehydrating the seaweed, for example, by solar drying followed by low heat finish drying and processing the dehydrated material into a granular meal or flour. A preferred seaweed meal is obtained from *Ascophyllum nodosum* and is available from Acadian Seaplants Limited of Nova Scotia, Canada, and is sold under the tradenames Acadian Kelp Meal and Tasco™-14. Acadian Kelp Meal and Tasco™-14 have the same composition. A typical analysis of Acadian Kelp Meal shows the following approximate weight percentages: moisture 12.0%, crude protein 6.0%, crude fiber 6.0%, ash (minerals) 22.0%, fat 20%, and carbohydrates 52.%. Analysis of Acadian Kelp Meal for carbohydrates gives by weight 18.0 - 27.0% alginic acid, 3.8 - 8.0% mannitol, 2.0 - 5.0% laminarin, and 20.0 - 22.0% other sugars. Analysis of Acadian Kelp Meal for minerals gives 50 - 150 ppm aluminum, 5 - 15 ppm barium, <1 ppm beryllium, 80 - 100 ppm boron, <1 ppm cadmium, 1.0 - 3.0% calcium, 1.0 - 3.0% chloride, 1 - 2 ppm chromium, <1 ppm cobalt, 1 - 10 ppm copper, <1,000 ppm iodine, 100 - 500 ppm iron, <1 ppm lead, 0.5 - 1.0% magnesium, 10-50 ppm manganese, <1 ppm mercury, <2 ppm molybdenum, <1 ppm nickel, 0.5 - 2.0% nitrogen, 0.1 - 0.2% phosphorus, 1.5 - 2.5% potassium, 3 - 4 ppm selenium, 2.4 - 4.0% sodium, 100 - 600 ppm strontium, 2.0 - 3.0% sulfur, <10 ppm tin, 1 - 10 ppm titanium, 2 - 6 ppm vanadium and 10 - 50 ppm zinc. Analysis of Acadian Kelp Meal for vitamins gives 0.1 - 0.4 ppm biotin, 30 - 60 ppm carotene, 0.1 - 0.5 ppm folic acid, 0.1 - 0.5 ppm folinic acid, 10 - 30 ppm niacin, 5 - 10 ppm riboflavin, I - 5 ppm thiamin, 150 - 300 ppm tocopherols, 100 - 2,000 ppm vitamin C,<0.004 ppm vitamin B_{12}, and <10 ppm vitamin K. Analysis of the amino acid content for Acadian Kelp Meal gave the following expressed as grams of amino acid per 100 g of protein nitrogen: alanine 5.3, arginine 8.0, aspartic acid 6.9, cystine (trace), glycine 5.0, glutamic acid 10.0, histidine 1.3, isoleucine 2.8, leucine 4.6, lysine 4.9, methionine 0.7, phenylalanine 2.3, proline 2.6, serine 3.0, threonine 2.8, tryptophan (trace), tyrosine 0.9, and valine 3.7.

[0022] We turn now to the embodiment of the invention in which seaweed supplement is directly fed to cattle during the feedlot finishing period of the life cycle of beef production. This embodiment is denoted the first embodiment.

[0023] The seaweed supplement for the first embodiment is that described above and preferably is seaweed extract obtained by extraction of *Ascophyllum nodosum.*

[0024] We turn now to feeding of the seaweed supplement for the first embodiment. It is included in diet in amount of, e.g., 0.01 to 5%, by weight, e.g., 0.1 to 3%, by weight (powder or concentrate extract or meal). Where the seaweed supplement is seaweed extract, the seaweed extract is preferably admixed into diet for direct feeding by inclusion at the time of feeding by top dressing or by mixing into the feed at the time of feeding or by premixing at the time the diet ingredients are combined. Where the seaweed supplement is seaweed meal, the seaweed meal can be admixed into diet by inclusion at the time the diet ingredients are mixed or by directed addition at the time of feeding.

[0025] As indicated above, for the first embodiment,

seaweed supplement is fed to cattle during the feedlot finishing period of the life cycle of beef production. The diet for feedlot finishing other than seaweed supplement can be one that is typical of feedlot finishing diets, e.g., a diet based on steamflaked milo (*Sorghum bicolor*) and cottonseed hulls (*Gossipium hirsutum*); however, the diet for feedlot finishing may also or alternatively be based on other ingredients including corn (*Zea mays*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) or other grains, especially corn. The feeding of seaweed supplement can be during the whole of the feedlot finishing period or part thereof, e.g., for at least 10 days of the feedlot finishing period. Preferably the feeding of seaweed supplement is for 10 to 20 days, e.g., for 14 days, at the end of the feedlot finishing period.

[0026] For the first embodiment herein, the slaughtering of cattle and obtaining meat therefrom can be carried out in conventional fashion.

[0027] For the first embodiment herein, analysis for *E. coli* can be carried out with the same frequency as is conventional for *E. coli* analysis on beef. For example, conventionally ground beef is tested in processing facilities for *E. coli* 0157:H7 on a random basis at least once quarterly, and carcasses at slaughter are checked for *E. coli* O157:H7 on a random basis at least once quarterly. If the presence of *E. coli* 0157:H7 is suspected, the testing could occur more frequently but there is no definite number of times. Analysis for pathogenic *E. coli* content of beef (in slaughtered carcasses and ground meat) can be carried out as described in Vanderzant, C., et al., Compendium of Methods of Microbiological Examination of Feed, 3rd edition, American Public Health Association, Chapter 24 (1992) or in Holt, J. G., Bergey's Manual of Determinative Bacteriology, 9th edition (1994).

[0028] We turn now to the embodiment of the invention in which seaweed supplement is incorporated on or into forage. This embodiment is denoted the second embodiment.

[0029] The forage for the second embodiment is pasture forage and can be any forage suitable for grazing of cattle. One important forage is tall fescue (*Festuca arundinacea* Schreb) which is grown on over 14 million hectares of land in the United States. Other forages include, for example, orchard grass (*Dactylis glomerata* L.), bluegrass (*Poa pratenisis L.*), bermudagrass (*Cynodon dactylon* L.), and ryegrass (*Lolium* spp.). The forage can be endophyte fungus infected. The fungus helps the plant tolerate stresses such as drought and insects. Endophyte fungus which infects tall fescue is *Neotyphodium coenophialum.* Endophyte fungus which infects ryegrass is *Acremonium lolii.*

[0030] The seaweed supplement of the second embodiment is that described above and preferably is seaweed extract obtained by extraction of *Ascophyllum nodosum.* When the seaweed supplement is seaweed extract, the seaweed extract is preferably applied to pasture forage as a water solution at the beginning of the grazing season and in the middle of the grazing season. The seaweed extract can be applied, for example, in an amount ranging from 0.3 kg/ha to 5 kg/ha, e.g., 1 to 4 kg/ha, and an application amount of 3.4 kg/ha (3 lbs/acre) has been used with good advantage. The seaweed extract (powder form) is readily dissolved in 20 to 40 gallons of water per acre (190 to 380 1/ha). Application is preferably carried out by spraying the water solution on the pasture forage using a commercial field-type of sprayer. When the seaweed supplement is seaweed meal, the seaweed meal is preferably applied to a pasture to provide seaweed treated forage by application in dry form and solubles from seaweed meal dissolve after application so that the solubilized material is available for foliar uptake and/or leaches into the ground and is taken up by the forage. The seaweed meal can be applied, for example, in an amount of 0.3 to 10 kg per "acre".

[0031] For the second embodiment, the cattle preferably are grazed on the seaweed extract treated pasture forage for 100 to 210 days (e.g., 180 to 200 days) in the spring and summer seasons in the year after birth and then are preferably feedlot finished over a period ranging from 75 to 200 days, e.g., 130 to 160 days. The feedlot finishing can be the same as that described for the first embodiment or can be conventional.

[0032] For the second embodiment herein, the slaughtering of cattle and obtaining meat therefrom can be carried out in conventional fashion.

[0033] For the second embodiment, analysis for E. *coli* can be carried out the same as for the first embodiment.

[0034] The invention is illustrated by the following working example.

Example

[0035] Forty-eight steers were fed a diet containing 0, 1% or 2% by weight Tasco-Ex (16 in each group) during the feedlot phase for two weeks before slaughter. The feedlot finishing phase constituted a period of about 120 days. Diets coded SER1, SER2 and SER3 were fed in succession for about two weeks each during the initial portion of the finishing period as the receiving and intermediate diets as the cattle were adjusted to the feedlot. After that and until the end of the feedlot phase, a diet denoted SER4 was fed and, as indicated above, for the last two weeks of the feedlot phase, the diet also contained 0%, 1% or 2% by weight Tasco-Ex. The SER1 diet consisted by weight of 44.7 steam flaked corn, 23.8% cottonseed hulls, 14.4% ground alfalfa hay, 2.21% supplement premix, 6.64% cottonseed meal, 0.55% urea, 2.5% fat, and 5.2% cane molasses. The SER2 diet consisted by weight of 56.7% steam flaked corn, 14.2% cottonseed hulls, 14.2% ground alfalfa hay, 0.82% urea, 3.73% cottonseed meal, 0.48% ground milo control, 2.17% supplement premix, 2.6% fat, and 5.1 % cane molasses. The SER3 diet consisted by weight of 65.4% steam flaked corn, 9.8% cottonseed hulls, 9.8% ground alfalfa hay, 3.9% cottonseed meal, 0.85% urea, 2.25% supplement premix, 2.7% fat, and 5.3% cane molasses.

The SER4 diet consisted by weight of 75.3% steam flaked corn, 4.9% ground alfalfa hay, 4.9% cottonseed hulls, 3.81 % cottonseed meal, 0.84% urea, 2.25% supplement premix, 2.7% fat, and 5.3% cane molasses. The supplement premix consisted by weight (dry matter basis) of 23.97% cottonseed meal, 42.11 % high-Ca limestone, 1.04% dicalcium phosphate, 8% potassium chloride, 3.56% magnesium oxide, 6.67% ammonium sulfate, 12% salt, 0.0018% cobalt carbonate, 0.16% copper sulfate, 0.13% iron sulfate, 0.0025% ethylenediaminedihydroiodide, 0.27% manganous oxide, 0.1% selenium premix (0.2%), 0.83% zinc sulfate, 0.012% vitamin A (650,000 International Units per kg), 0.126% vitamin E (500 International Units per kg), 0.67% Rumensin-80 (80 grams rumensin per lb. of Rumensin-80), and 0.36% Tylan-40 (40 grams of tylan per lb. of Tylan-40).

[0036] Slaughter was carried out and analyses for E. *coli* (all strains) and for E. *coli* strain 0157:H7 were carried out on fecal and hide samples from all the steers, both before and after slaughter. In the analyses, one (1) was assigned if E. *coli* was absent and two (2) was assigned if E. *coli* was present and the results for each group were averaged.

[0037] Analyses on fecal samples were carried out as follows:

[0038] Twenty grams of fecal sample is collected and taken to the laboratory within 4 hours. Ten grams of each fecal sample is incubated with enrichment medium for 6 hours at 37°C. Following the incubation period, 1 ml of enriched sample is added to 20 μl Dynal anti-0157 magnetic beads (Dynal Corp.) in a capped test tube and incubated at room temperature for 30 min. Dyna-bead bacterial complex is re-suspended in wash buffer and plated on McConkey sorbital agar + cefixime and potassium telunite, and incubated for 24 hours at 37°C. O agglutination (0157) tests are performed on typical colonies (clear colonies). Agglutinating colonies are inoculated on tryptic soy agar (TSA) and motility plates and incubated for 24 hrs at 35°C and 32°C, respectively. Colonies collected from motility plates are used to inoculate brain and heart infusion (BHI tubes that are incubated at 32°C for 24 hrs). Colonies isolated from motility and O agglutination tests are transferred to API 20E test strips (BioMerieux Vitek, St. Louis, Missouri) and read according to manufacturers recommendations. H agglutination (H7) tests are performed using a sample of the brain and heart infusion (BHI) broth. These combined procedures allow the determination of the species and pathogenicity of detected *E. coli.*

[0039] Analyses on hide samples were carried out as follows: The same procedure was used as on fecal samples, except that instead of 20 grams of fecal sample being collected, a sponge was used to swipe the hide and then the presence of organisms on the sponge was determined using the same procedures recited above for analysis in respect to fecal samples.

[0040] The results for E. *coli* (all strains) on fecal samples are shown in Fig. 1. As shown in Fig 1, a linear effect (decrease in fecal *E. coli* content with Tasco-Ex treatment) was obtained (P<0.02). The results on fecal samples for *E. coli* (strain 0157:H7) are shown in Fig. 3. As shown in Fig. 3, no 0157:H7 was found in the groups treated with Tasco-Ex and the control differed from the mean of the treatments (P<0.11).

[0041] The results for E. *coli* (all strains) on hide samples are shown in Fig. 2. Fig. 2 shows decrease in hide E. *coli* with Tasco-Ex treatment. The results for E. *coli* (strain O157:H7) are shown in Fig. 4. As shown in Fig. 4, no O157:H7 was found in the groups treated with Tasco-Ex and the control differed from the mean of the treatments (P<0.02).

[0042] In data using an enumeration method, the actual levels of coliforms and E. *coli,* in the feces as enumerated with E. *coli* Petrifilm™, were not different among treatments (P<0.05); this data does not detract from a conclusion based on the data of Figs. 1-4 that seaweed supplement administration causes reduced pathogenic E. *coli* content in beef and reduced *E. coli* content in general (since the procedures used for the data of Figs. 1 and 2 were more sensitive for *E. coli* in general than were the procedures of the enumeration method).

[0043] After slaughter analysis on samples of meat show pathogenic *E. coli* reduction in meat from steers treated with seaweed supplement.

[0044] Similar results of reduced pathogenic *E. coli* content in beef are obtained when cattle graze on pasture forage (endophyte infected tall fescue) treated with Tasco-Ex at 3 1b/acre (3.4 kg/ha) in the beginning at in the middle of the grazing season and seaweed supplement is fed or is not fed during feedlot finishing.

Claims

1. The use of seaweed in the manufacture of a supplement for use during feedlot finishing period of the life cycle of beef production and/or incorporated on or into forage during cattle grazing to reduce the pathogenic E. coli content of meat obtained on slaughter of the cattle.

2. A use of Claim 1, wherein the supplement is for use during feedlot finishing period of the life cycle of beef production.

3. A use of Claim 2, where the supplement is for feeding for 10 to 20 days at the end of the feedlot finishing period.

4. A use of Claim 2 or Claim 3, where the supplement is for feeding in an amount ranging from 0.01 to 5% by weight of diet.

5. A use of Claim 1, wherein the supplement is for use incorporation on or into forage during cattle grazing.

6. A use of Claim 5, wherein the forage is tall fescue grass.

7. A use of Claim 5 or Claim 6, wherein the supplement is a seaweed extract for incorporated by application onto the forage.

8. A use of Claim 7, wherein the seaweed extract is for application onto the forage in an amount of 0.3 kg/ha to 5 kg/ha.

9. A use of any one of Claims 6 to 8, wherein the tall fescue grass is infected with an endophyte fungus.

10. A use of Claim 9, wherein the endophyte fungus is *Neotyphodium coenophialum.*

11. A use of any one of the preceding claims, where the supplement is water-soluble seaweed extract obtained from alkaline hydrolysis of seaweed.

12. A use of any one of Claims 1 to 6, 9 and 10, where the supplement is seaweed meal or flour obtained from dehydrated seaweed.

13. A use of any one of the preceding claims, where the seaweed supplement is obtained from *Ascophyllum nodosum*

14. A use of any one of the preceding claims, where said pathogenic *E. coli* comprises *E. coli* 0157:H7.

15. A method of obtaining beef of reduced content of *E. coli* O157:H7 comprising the steps of:

(a) directly feeding seaweed supplement to cattle during the feedlot finishing period of the life cycle of beef production;
(b) slaughtering the cattle and obtaining meat; and
(c) analyzing for *E. coli* 0157:H7 content in samples of the meat;

thereby to obtain and demonstrate the presence of beef of reduced *E. coli* O157:H7 content compared to if seaweed supplement were not fed.

16. A method of obtaining beef of reduced *E. coli* O157:H7 content comprising the steps of:

(i) slaughtering cattle directly fed on seaweed supplement during the feedlot finishing period of the life cycle of beef production and obtaining meat; and
(ii) analyzing for *E. coli* O157:H7 content in samples of the meat;

thereby to obtain and demonstrate the presence of beef of reduced *E. coli* 0157:H7content compared to if seaweed supplement were not fed.

17. A method of Claim 15 or Claim 16, where the seaweed supplement is fed for 10 to 20 days at the end of the feedlot finishing period.

18. A method of any one of Claims 15 to 17, where the seaweed supplement is fed in an amount ranging from 0.01 to 5% by weight of diet.

19. A method of obtaining beef of reduced content of *E. coli* 0157:H7 comprising the steps of:

(a) grazing cattle on forage on or into which seaweed supplement has been incorporated;
(b) slaughtering the cattle and obtaining meat; and
(c) analyzing for *E. coli* 0157:H7 content in samples of the meat;

thereby to obtain and demonstrate the presence of beef of reduced *E. coli* 0157:H7 content compared to if seaweed supplement were not fed.

20. A method of obtaining beef of reduced *E. coli* O157:H7 content comprising the steps of:

(i) slaughtering cattle grazed on forage on or into which seaweed supplement has been incorporated and obtaining meat; and
(c) analyzing for *E. coli* O157:H7 content in samples of the meat;

thereby to obtain and demonstrate the presence of beef of reduced *E. coli* 0157:H7 content compared to if seaweed supplement were not fed.

21. A method of Claim 19 or Claim 20, wherein the forage is tall fescue grass.

22. A method of any one of Claims 19 to 21, wherein the seaweed supplement is incorporated by application of seaweed extract onto the forage.

23. A method of Claim 22, wherein the seaweed extract is applied onto the forage in an amount of 0.3 kg/ha to 5 kg/ha.

24. A method of any one of Claims 21 to 23, wherein the tall fescue grass is infected with an endophyte fungus.

25. A method of Claim 24, wherein the endophyte fungus is *Neotyphodium coenophialum.*

26. The use of seaweed supplement during feedlot finishing period of the life cycle of beef production

and/or incorporated on or into forage during cattle grazing to reduce the content in meat obtained on slaughter of the cattle of *E. coli* 0157:H7.

Patentansprüche

1. Die Verwendung von Meeresalgen bei der Herstellung eines Zusatzes zur Verwendung während der Feedlot-Endperiode im Lebenszyklus der Rindfleischproduktion und/oder welcher während des Weidens der Rinder auf oder in das Futter auf- bzw. eingebracht wird, um den Gehalt pathogener *E. coli* in Fleisch, das beim Schlachten der Rinder erhalten wird, zu verringern.

2. Verwendung nach Anspruch 1, wobei der Zusatz zur Verwendung während der Feedlot-Endperiode im Lebenszyklus der Rindfleischproduktion dient.

3. Verwendung nach Anspruch 2, wobei der Zusatz zum Füttern für 10 bis 20 Tage am Ende der Feedlot-Endperiode dient.

4. Verwendung nach Anspruch 2 oder Anspruch 3, wobei der Zusatz zum Füttern in einer Menge, die von 0,01 bis 5 Gew.-% der Nahrung (diet) reicht, dient.

5. Verwendung nach Anspruch 1, wobei der Zusatz zur Verwendung bei der Auf- bzw. Einbringung auf oder in das Futter während des Weidens der Rinder dient.

6. Verwendung nach Anspruch 5, wobei das Futter Rohrschwingelgras ist.

7. Verwendung nach Anspruch 5 oder Anspruch 6, wobei der Zusatz ein Meeresalgenextrakt für eine Auf- bzw. Einbringung durch Auftragung auf das Futter ist.

8. Verwendung nach Anspruch 7, wobei der Meeresalgenextrakt zur Auftragung auf das Futter in einer Menge von 0,3 kg/ha bis 5 kg/ha dient.

9. Verwendung nach irgendeinem der Ansprüche 6 bis 8, wobei das Rohrschwingelgras mit einem Endophytenpilz infiziert ist.

10. Verwendung nach Anspruch 9, wobei der Endophytenpilz *Neotyphodium coenophialum* ist.

11. Verwendung nach irgendeinem der vorhergehenden Ansprüche, wobei der Zusatz ein wasserlöslicher Meeresalgenextrakt ist, welcher aus einer alkalischen Hydrolyse von Meeresalgen erhalten wird.

12. Verwendung nach irgendeinem der Ansprüche 1 bis 6, 9 und 10, wobei der Zusatz Meeresalgenpulver oder -mehl ist, das aus entwässerten Meeresalgen erhalten wird.

13. Verwendung nach irgendeinem der vorhergehenden Ansprüche, wobei der Meeresalgenzusatz aus *Aseophyllum nodosum* erhalten wird.

14. Verwendung nach irgendeinem der vorhergehenden Ansprüche, wobei der pathogene *E. coli E. coli* O157:H7 umfasst.

15. Ein Verfahren zum Erhalt von Rindfleisch mit einem verringerten Gehalt an *E. coli* O157:H7, welches die folgenden Schritte umfasst:

    (a) direktes Füttern von Meeresalgenzusatz an Rinder während der Feedlot-Endperiode im Lebenszyklus der Rindfleischproduktion;
    (b) Schlachten der Rinder und Erhalt des Fleisches; und
    (c) Analysieren auf den *E. coli* O157: H7-Gehalt in Proben des Fleisches;

    um **dadurch** das Vorliegen von Fleisch mit verringertem *E. coli* O157:H7-Gehalt im Vergleich dazu, wenn der Meeresalgenzusatz nicht gefüttert wird, zu demonstrieren und dieses zu erhalten.

16. Ein Verfahren zum Erhalt von Rindfleisch mit einem verringerten Gehalt an *E. coli* 0157:H7, welches die folgenden Schritte umfasst:

    (i) Schlachten von Rindern, welche direkt während der Feedlot-Endperiode im Lebenszyklus der Rindfleischproduktion mit Meeresalgenzusatz gefüttert wurden, und Erhalt des Fleisches; und
    (ii) Analysieren auf den *E. coli* O157:H7-Gehalt in Proben des Fleisches;

    um **dadurch** das Vorliegen von Fleisch mit verringertem *E. coli* O1 57:H7-Gehalt im Vergleich dazu, wenn der Meeresalgenzusatz nicht gefüttert wird, zu demonstrieren und dieses zu erhalten.

17. Verfahren nach Anspruch 15 oder Anspruch 16, wobei der Meeresalgenzusatz für 10 bis 20 Tage am Ende der Feedlot-Endperiode gefüttert wird.

18. Verfahren nach irgendeinem der Ansprüche 15 bis 17, wobei der Meeresalgenzusatz in einer Menge, die von 0,01 bis 5 Gew.% der Nahrung reicht, gefüttert wird.

19. Ein Verfahren zum Erhalt von Rindfleisch mit einem verringerten Gehalt an *E. coli* 0157:H7, welches die folgenden Schritte umfasst:

(a) Weiden lassen von Rindern auf Futter, auf oder in welches ein Meeresalgenzusatz auf- bzw. eingebracht wurde;
(b) Schlachten der Rinder und Erhalt des Fleisches; und
(c) Analysieren auf den *E. coli* O157:H7-Gehalt in Proben des Fleisches;

um **dadurch** das Vorliegen von Fleisch mit verringertem *E. coli* O157:H7-Gehalt im Vergleich dazu, wenn der Meeresalgenzusatz nicht gefüttert wird, zu demonstrieren und dieses zu erhalten.

20. Ein Verfahren zum Erhalt von Rindfleisch mit einem verringerten Gehalt an *E. coli* 0157:H7, welches die folgenden Schritte umfasst:

(i) Schlachten von Rindern, welche auf Futter geweidet wurden, auf oder in welches Meeresalgenzusatz auf- bzw. eingebracht wurde, und Erhalt des Fleisches; und
(c) Analysieren auf den *E. coli* 0157:H7-Gehalt in Proben des Fleisches; um **dadurch** das Vorliegen von Fleisch mit verringertem *E. coli* O157:H7-Gehalt im Vergleich dazu, wenn der Meeresalgenzusatz nicht gefüttert wird, zu demonstrieren und dieses zu erhalten.

21. Verfahren nach Anspruch 19 oder Anspruch 20, wobei das Futter Rohrschwingelgras ist.

22. Verfahren nach irgendeinem der Ansprüche 19 bis 21, wobei der Meeresalgenzusatz durch Auftragung von Meeresalgenextrakt auf das Futter auf- bzw. eingebracht wird.

23. Verfahren nach Anspruch 22, wobei der Meeresalgenextrakt auf das Futter in einer Menge von 0,3 kg/ha bis 5 kg/ha aufgetragen wird.

24. Verfahren nach irgendeinem der Ansprüche 21 bis 23, wobei das Rohrschwingelgras mit einem Endophytenpilz infiziert ist.

25. Verfahren nach Anspruch 24, wobei der Endophytenpilz *Neotyphodium coenophialum* ist.

26. Die Verwendung eines Meeresalgenzusatzes während der Feedlot-Endperiode im Lebenszyklus der Rindfleischproduktion und/oder welcher während des Weidens der Rinder auf oder in das Futter auf- bzw. eingebracht wird, um den Gehalt in Fleisch, das beim Schlachten der Rinder erhalten wird, an *E. coli* O157: H7 zu verringern.

Revendications

1. Utilisation d'algues dans la fabrication d'un supplément utile pendant la période de finition de l'engraissement du cycle de vie de la production de boeuf de boucherie et/ou incorporées sur ou dans le fourrage pendant le pacage du bétail pour réduire le taux d'*E. coli* pathogène dans la viande obtenue après abattage du bétail.

2. Utilisation selon la revendication 1, dans laquelle le supplément est destiné à être utilisé pendant la période de finition de l'engraissement du cycle de vie de la production de boeuf de boucherie.

3. Utilisation selon la revendication 2, dans laquelle le supplément est destiné à l'alimentation animale pendant 10 à 20 jours à la fin de la période de finition de l'engraissement.

4. Utilisation selon la revendication 2 ou la revendication 3, dans laquelle le supplément est destiné à être utilisé pour l'alimentation animale à un taux allant de 0,01 à 5 % en masse par rapport à la ration.

5. Utilisation selon la revendication 1, dans laquelle le supplément est destiné à être utilisé, incorporé sur ou dans le fourrage, pendant le pacage du bétail.

6. Utilisation selon la revendication 5, dans laquelle le fourrage est de la fétuque élevée.

7. Utilisation selon la revendication 5 ou la revendication 6, dans laquelle le supplément est un extrait d'algues à incorporer par application sur le fourrage.

8. Utilisation selon la revendication 7, dans laquelle l'extrait d'algues est destiné à une application sur le fourrage en une quantité de 0,3 kg/ha à 5 kg/ha.

9. Utilisation selon l'une quelconque des revendications 6 à 8, dans laquelle la fétuque élevée est infectée par un champignon endophyte.

10. Utilisation selon la revendication 9, dans laquelle le champignon endophyte est *Neotyphodium coenophialum.*

11. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle le supplément est un extrait d'algues soluble dans l'eau obtenu par hydrolyse alcaline d'algues.

12. Utilisation selon l'une quelconque des revendications 1 à 6, 9 et 10, dans laquelle le supplément est une farine d'algues en granulés ou en poudre obtenue à partir d'algues déshydratées.

13. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle le supplément à base d'algues est obtenu à partir *d'Ascophyllum nodosum.*

14. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle ledit *E. coli* pathogène comprend *E. coli* 0157:H7.

15. Procédé d'obtention de boeuf ayant un taux réduit d'*E. coli* 0157:H7 comprenant les étapes de :

(a) l'apport direct dans l'alimentation du bétail d'un supplément à base d'algues pendant la période de finition de l'engraissement du cycle de vie de la production de boeuf de boucherie ;
(b) l'abattage du bétail et l'obtention de viande ; et
(c) l'analyse du taux d'*E. coli* O 157: H7 dans les échantillons de viande ;

pour obtenir ainsi et démontrer la présence de boeuf ayant un taux réduit d'*E. coli* 0157:H7 par rapport au cas où l'on n'utilise pas de supplément à base d'algues dans l'alimentation.

16. Procédé d'obtention de boeuf ayant un taux réduit d'*E. coli* O157:H7 comprenant les étapes de :

(i) l'abattage du bétail directement alimenté sur un supplément à base d'algues pendant la période de finition de l'engraissement du cycle de vie de la production de boeuf de boucherie et l'obtention de viande ; et
(ii) l'analyse du taux *d'E. coli* O157:H7 dans les échantillons de viande ;

pour obtenir ainsi et démontrer la présence de boeuf ayant un taux réduit d'*E. coli* 0157:H7 par rapport au cas où l'on n'utilise pas de supplément à base d'algues.

17. Procédé selon la revendication 15 ou 16, dans lequel le supplément à base d'algues est apporté dans l'alimentation pendant 10 à 20 jours à la fin de la période de finition de l'engraissement.

18. Procédé selon l'une quelconque des revendications 15 à 17, dans lequel le supplément à base d'algues est apporté dans l'alimentation à un taux allant de 0,01 à 5 % en masse par rapport à la ration.

19. Procédé d'obtention de boeuf ayant un taux réduit *d'E. coli* 0157:H7 comprenant les étapes :

(a) de pacage du bétail sur du fourrage sur ou dans lequel a été incorporé un supplément à base d' algues ;
(b) de l'abattage du bétail et de l'obtention de viande ; et
(c) de l'analyse du taux *d'E. coli* O157:H7 dans les échantillons de viande ;

pour obtenir ainsi et démontrer la présence de boeuf ayant un taux réduit *d'E. coli* O157:H7 par rapport au cas où l'on n'utilise pas de supplément à base d'algues.

20. Procédé d'obtention de boeuf ayant un taux réduit d'*E. coli* O157:H7 comprenant les étapes de :

(i) l'abattage du bétail nourri au pâturage sur du fourrage sur ou dans lequel a été incorporé un supplément à base d' algues ;
(ii) l'analyse du taux d*'E. coli* O157:H7 dans les échantillons de viande ;

pour obtenir ainsi et démontrer la présence de boeuf ayant un taux réduit d'*E. coli* O157:H7 par rapport au cas où l'on n'utilise pas de supplément à base d'algues.

21. Procédé selon la revendication 19 ou 20, dans lequel le fourrage est de la fétuque élevée.

22. Procédé selon l'une quelconque des revendications 19 à 21, dans lequel le supplément à base d'algues est incorporé par application d'extrait d'algues sur le fourrage.

23. Procédé selon la revendication 22, dans lequel l'extrait d'algues est appliqué sur le fourrage en une quantité de 0,3 kg/ha à 5 kg/ha.

24. Procédé selon l'une quelconque des revendications 21 à 23, dans lequel la fétuque élevée est infectée par un champignon endophyte.

25. Procédé selon la revendication 24, dans lequel le champignon endophyte est *Neotyphodium coenophialum.*

26. Utilisation d'un supplément à base d'algues pendant la période de finition de l'engraissement du cycle de vie de la production de boeuf de boucherie et/ou incorporé sur ou dans le fourrage pendant le pacage du bétail pour réduire le taux d'*E. coli* O157:H7 dans la viande obtenue après abattage du bétail.

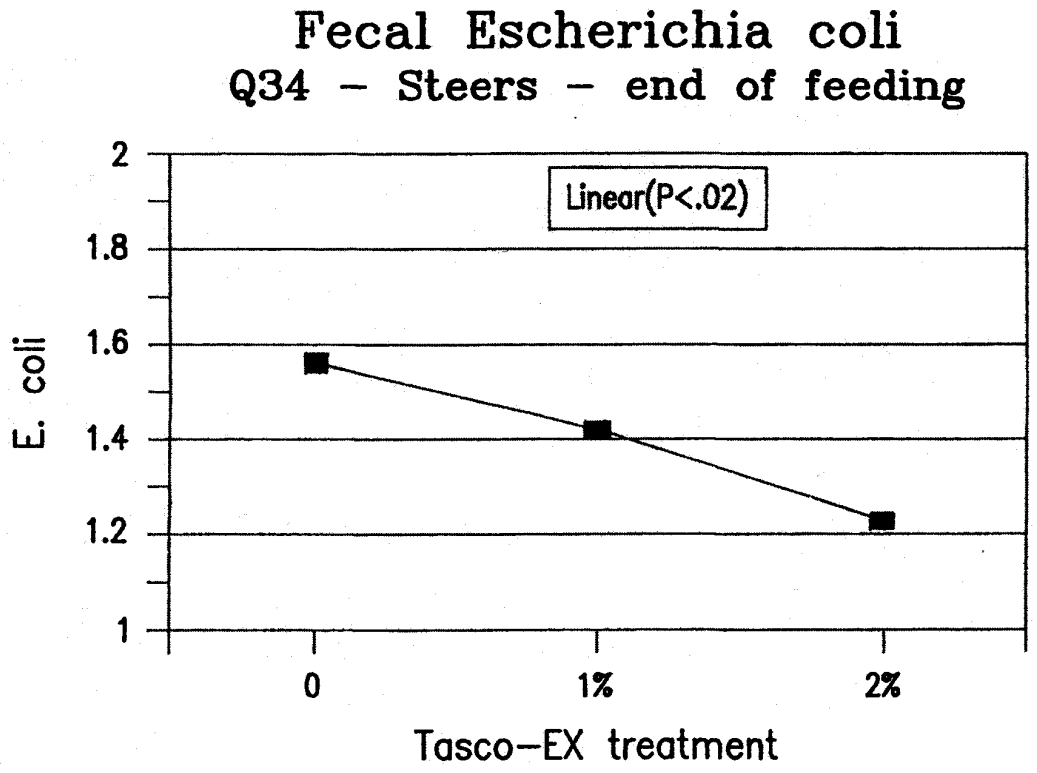
Fecal Escherichia coli
Q34 – Steers – end of feeding
Linear(P<.02)
E. coli
2
1.8
1.6
1.4
1.2
1
0
1%
2%
Tasco–EX treatment

FIG. 1

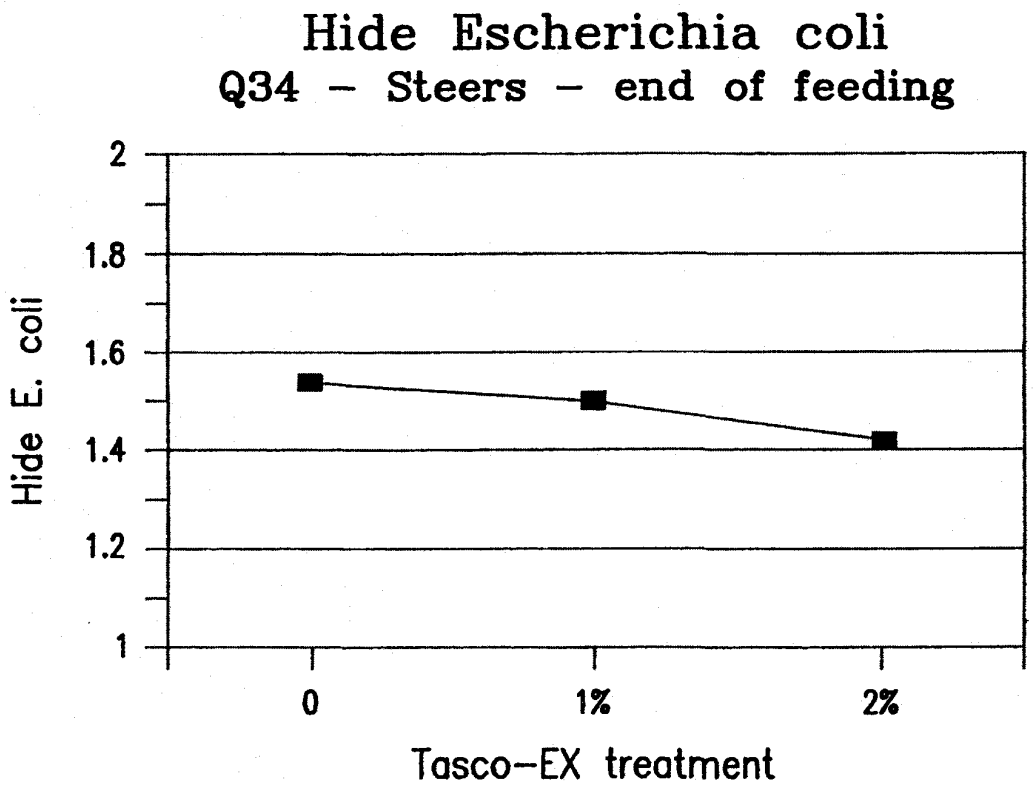
Hide Escherichia coli
Q34 – Steers – end of feeding
Hide E. coli
2
1.8
1.6
1.4
1.2
1
0
1%
2%
Tasco–EX treatment

FIG. 2

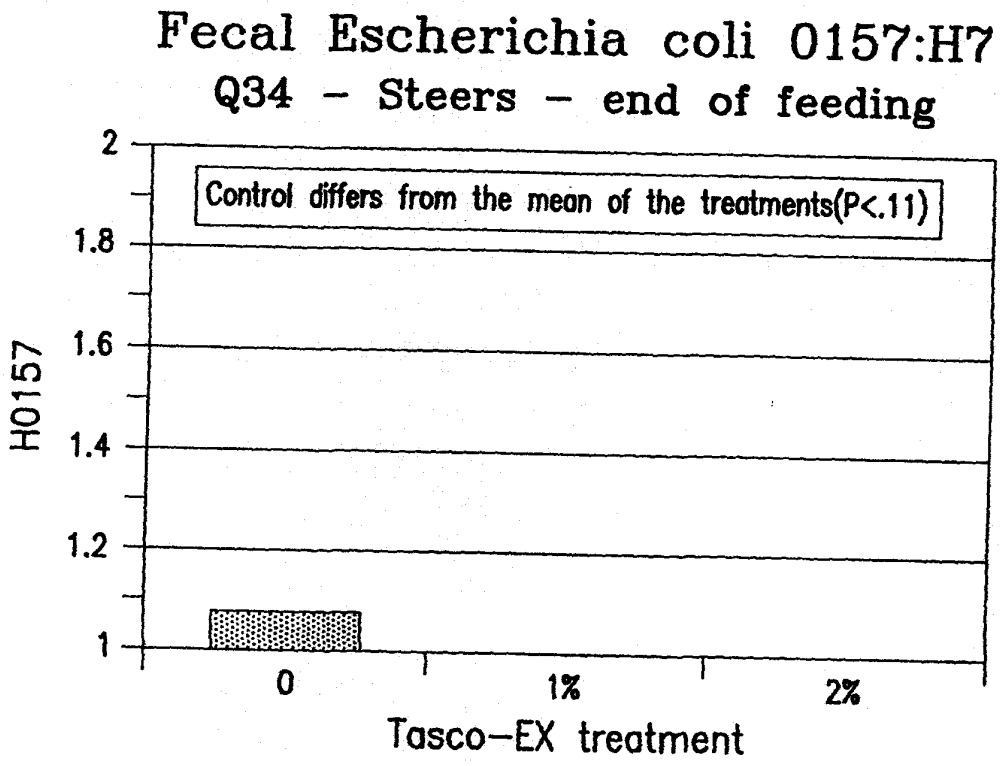
Fecal Escherichia coli 0157:H7
Q34 - Steers - end of feeding
Control differs from the mean of the treatments(P<.11)
H0157
2
1.8
1.6
1.4
1.2
1
0
1%
2%
Tasco-EX treatment

FIG. 3

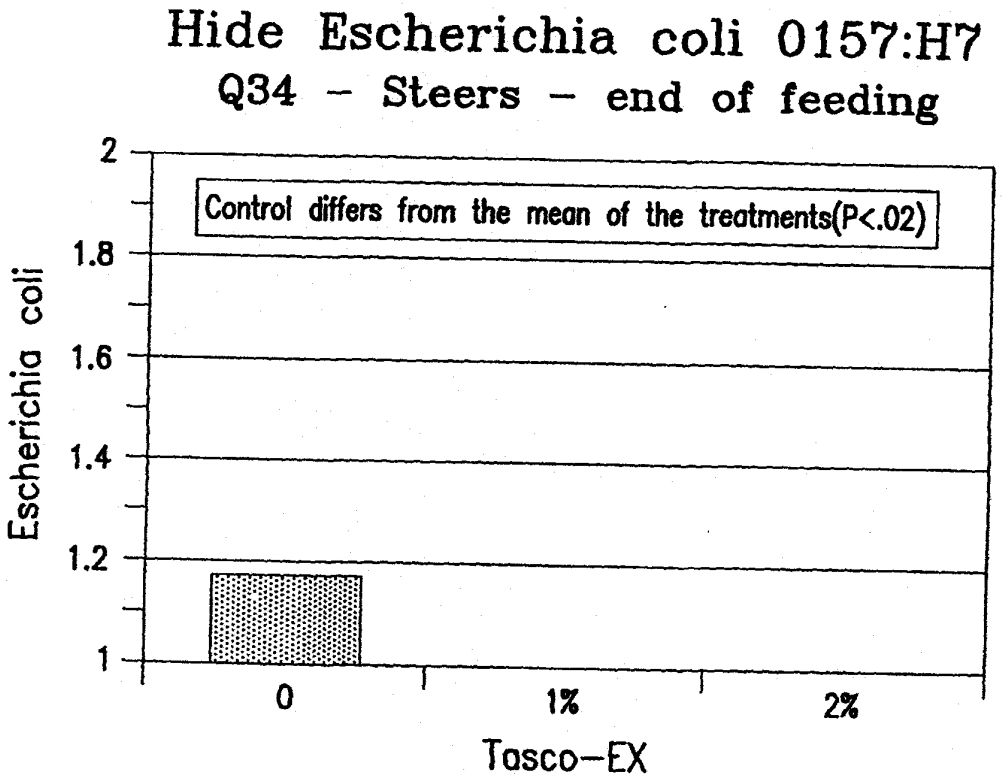
Hide Escherichia coli 0157:H7
Q34 - Steers - end of feeding
Control differs from the mean of the treatments(P<.02)
Escherichia coli
2
1.8
1.6
1.4
1.2
1
0
1%
2%
Tasco-EX

FIG. 4

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **VANDERZANT, C et al.** Compendium of Methods of Microbiological Examination of Feed. American Public Health Association, 1992 **[0027]**
- **HOLT, J. G.** Bergey's Manual of Determinative Bacteriology. 1994 **[0027]**