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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

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(54) Title: TALL OIL FATTY ACID FOR USE IN TREATMENT AND ANIMAL FEED SUPPLEMENTS AND COMPOSITIONS

(57) Abstract: The present invention relates to a tall oil fatty acid for use in the prevention of growth of harmful bacteria in the animal digestive tractand/or in the prevention of intestinal disorders. The invention further relates to a feed supplement and a feed composition comprising tall oil fatty acid.

WO 2014/184430 A1

TALL OIL FATTY ACID FOR USE IN TREATMENT AND ANIMAL FEED SUPPLEMENTS AND COMPOSITIONS

FIELD OF THE INVENTION

The invention relates to a tall oil fatty acid, use thereof, and feed supplement and feed composition comprising said tall oil fatty acid.

BACKGROUND OF THE INVENTION

Imbalances in microbial populations and growth of harmful bacteria in the digestive tract of animals can cause significant losses in animal growth and production. These imbalances manifest themselves as intestinal disorders such as diarrhea. While microbial infections of animals have been prevented by the use of e.g. antibiotics and other agents that prevent the growth of microorganisms, stricter regulations on their use are expected. Generally, there is an increasing demand for ingredients for use in animal feeding that can modulate the microbial population in the animal digestive tract but which are readily available, well tolerated and environmentally friendly.

Fractional distillation of crude tall oil, obtained as a by-product of the Kraft process of wood pulp manufacture, produces distilled tall oil (DTO) which typically comprises over 10% resin acids and less than 90% fatty acids. Further refinement of distilled tall oil produces tall oil fatty acid (TOFA), which is available in a variety of compositions differing in the fatty acids and resin acids content. Because TOFA is an inexpensive source of fatty acids, it has previously been used in animal nutrition as an energy source. For instance, GB 955316 discloses the use of alkali metal salts of tall oil fatty acids to improve weight gain and nitrogen retention in ruminant animals.

PURPOSE OF THE INVENTION

The purpose of the invention is to provide a new type of tall oil fatty acid/feed supplement for use in the prevention of growth of harmful bacteria in the animal digestive tract and/or in the prevention of intestinal disorders.

The present inventors have surprisingly found that TOFA prevents the growth of harmful bacteria in the animal digestive tract and/or prevents intestinal disorders.

SUMMARY

The tall oil fatty acid according to the present invention is characterized by what is presented in claim 1.

The feed supplement according to the present invention is characterized by what is presented in claim 7.

The feed composition according to the present invention is characterized by what is presented in claim 12.

DETAILED DESCRIPTION OF THE INVENTION

FIG 1a The turbidity change during 8 hours of Cl. perfringens growth as a response to TOFA and digested TOFA concentrations.

FIG 1b Gas production during 8 hours by *Cl. perfringens* growth as a response to TOFA and digested TOFA concentrations.

FIG 2a The turbidity change during 8 hours of Cl. perfringens growth as a response to TOFA and test products at dose 1.

FIG 2b The turbidity change during 8 hours of Cl. perfringens growth as a response to TOFA and test products at dose 2.

FIG 2c The turbidity change during 8 hours of *S. aureus* as a response to TOFA and test products at dose 1.

FIG 2d The turbidity change during 8 hours of *S. aureus* as a response to TOFA and test products at dose 2.

FIG 2e The turbidity change during 8 hours of *S. suis* as a response to TOFA and test products at dose 1.

FIG 2f The turbidity change during 8 hours of *C S. suis* as a response to TOFA and test products at dose 2.

The present invention is based on the realization that tall oil fatty acid can be used in the prevention of growth of harmful bacteria in the animal digestive tract and/or in the prevention of intestinal disorders.

The term "tall oil fatty acid" or "TOFA" should be understood as referring to a composition obtained by distillation of crude tall oil and further refinement of distilled tall oil. TOFA typically comprises 90-98% (w/w) fatty acids. Further, TOFA may comprise 1-10% (w/w) resin acids.

In one embodiment of the present invention, the tall oil fatty acid for use according to the present invention comprises 1-10% (w/w) of resin acids.

In one embodiment of the present invention, TOFA comprises 2-9 % (w/w) resin acids.

In one embodiment of the present invention, TOFA comprises 5-9% (w/w) resin acids.

In this context, the term "resin acids" should be understood as referring to a complex mixture of various acidic compounds comprised by tall oil which share the same basic skeleton including a three-fused ring. The exact composition of the resin acids present in TOFA varies e.g. according to the species of the trees the TOFA is obtained from and the pro-

cessing conditions under which it is manufactured. Resin acids typically include compounds such as abietic acid, dehydroabietic acid, levopimaric acid, neoabietic acid, pimaric acid and isopimaric acid, only to mention a few.

In one embodiment of the present invention, TOFA comprises 90-98% (w/w) of fatty acids.

The tall oil fatty acid (TOFA) is produced by refinement from distilled tall oil. Distilled tall oil (DTO) is produced by fractional distillation from crude tall oil, obtained as a by-product of the Kraft process of wood pulp manufacture.

In one embodiment of the present invention, the TOFA, for use according to the present invention is dried. The TOFA can be dried by spray drying, drum drying or by any other known suitable drying method.

The present invention also relates to a feed supplement comprising the tall oil fatty acid according to the invention.

In one embodiment of the present invention, the feed supplement is effective in the prevention of growth of harmful bacteria, for prevention of intestinal disorders, in the modulation of microbial population of the animal digestive tract, in enhancing rumen fermentation and/or lowering rumen methane production.

In one embodiment of the present invention, the feed supplement comprises a tall oil fatty acid which comprises 1-10% (w/w) resin acids.

In one embodiment of the present invention, the feed supplement comprises a tall oil fatty acid which comprises 2-9 % (w/w) resin acids.

In one embodiment of the present invention, the feed supplement comprises a tall oil fatty acid which comprises 5-9% (w/w) resin acids.

In this context, the term “feed supplement” should be understood as referring to a composition that may be added to a feed or used as such in the

feeding of animals. The feed supplement may comprise different active ingredients. The feed supplement may be added in the feed in a concentration of 0.0001 – 5 kg//ton of dry weight, preferably 0.005 – 1 kg/ton of the dry weight of the total amount of the feed. The TOFA or the feed supplement comprising the TOFA according to the invention may be added to the feed or feed supplement as such, or it may in general be further processed as desired.

Further, the TOFA or the feed supplement comprising the TOFA according to the invention may be added to the feed or feed supplement, or it may be administered to an animal separately (i.e. not as a part of any feed composition).

In this context, the term "feed composition" or "feed" should be understood as referring to the total feed composition of an animal diet or to a part thereof, including e.g. supplemental feed, premixes and other feed compositions. The feed may comprise different active ingredients.

In one embodiment of the present invention, the feed supplement comprises TOFA which is absorbed into a carrier material suitable for the feed composition such as sugarbeet pulp.

In one embodiment of the present invention, the feed supplement comprises TOFA which is dried.

The present invention also relates to a feed composition comprising the feed supplement according to the invention.

In one embodiment of the present invention, the feed composition comprises the feed supplement in an amount of 0.00001 – 0.5 % (w/w), of the dry weight of the total amount of the feed.

In one embodiment of the present invention, the feed composition comprises the feed supplement in an amount of 0.0005 – 0.1 % (w/w) of the dry weight of the total amount of the feed.

In one embodiment of the present invention, the method of producing a tall oil fatty acid or feed supplement further comprises a step of drying. The dying can be carried out by spray drying, drum drying or by any other known drying method.

The invention also relates to a method of preventing the growth of harmful bacteria in the animal digestive tract and/or preventing intestinal disorders, comprising the step of administering to an animal the tall oil fatty acid according to the invention.

In this context, the term "harmful bacteria" should be understood as referring to any bacteria that is capable of affecting the digestive tract or health of an animal in an adverse manner, including competition for nutrients with the host animal. In this context, the term "microbial population" should be understood as referring to the microorganisms that inhabit the digestive tract, including the Bacteria and Archaea domains and microscopic members of the Eukaryote domain and also intestinal parasites. The microbial population will vary for different animal species depending on e.g. the health of an animal and on environmental factors.

In this context, the term "intestinal disorder" should be understood as referring to various disorders of the digestive tract in an animal, including e.g. diarrhea and other intestinal health problems.

In this context, the term "animal" should be understood as referring to all kinds of different animals, such as monogastric animals, ruminants, fur animals, pets and aquaculture. Non-limiting examples of different animals, including offspring, are cows, beef cattle, pigs, poultry, sheep, goats, horses, foxes, dogs, cats and fish.

In one embodiment of the present invention, the TOFA is administered to an animal in an effective

amount. In a further embodiment, the TOFA is administered in a therapeutically effective amount.

The present invention has a number of advantages. TOFA is a readily available, natural, low-cost and environmentally friendly material. Further, it is non-toxic and well tolerated. Subsequently, other benefits of the invention are e.g. improved animal health and productivity, higher product quality, uniformity, food and product safety. The invention also allows the production of feed compositions and supplements at low cost.

The embodiments of the invention described hereinbefore may be used in any combination with each other. Several of the embodiments may be combined together to form a further embodiment of the invention. A product, a method or a use, to which the invention is related, may comprise at least one of the embodiments of the invention described hereinbefore.

EXAMPLES

In the following, the present invention will be described in more detail.

EXAMPLE 1.

Pathogen inhibition test

Clostridium perfringens is a pathogenic bacterium that causes necrotic enteritis in broiler chicks and other species of poultry. This experiment was conducted to study the inhibition of *Cl. perfringens* by TOFA with 5 % resin acids.

The efficiency of untreated and digested test compounds was tested in a *Cl. perfringens* growth inhibition test that measures both the turbidity of the clostridial culture medium as a result of increased number of bacterial cells in a unit volume of medium,

and the cumulative gas production during the simulation.

There were four treatments in the test: control, control/ethanol, TOFA 5 % and pre-digested TOFA 5% and the TOFA products were tested in two concentrations. To make the untreated TOFA 5% soluble in the water phase of the simulation medium, it was first diluted with ethanol. The digested TOFA product was diluted in sterile water.

Gastrointestinal digestion of the TOFA: The tall oil was digested in 5% and 1% stock solutions, starting with pepsin-HCl digestion (pH 2.5) at +37°C treatment for 3 hours, followed by neutralization of the digesta with NaOH (pH 6.5) and the treatment with bile acids and pancreatin for additional 3 hours at +37°C. This digestion mimics the gastric and small-intestinal digestion of monogastric animals. Digestion was made to evaluate whether the product would resist the conditions of upper gastrointestinal tract before entering to the distal intestine with higher microbial activity.

The simulation was conducted in 25-ml glass bottles containing 15 ml of sterile anaerobic TSGY-media (tryptic soy broth -yeast extract media with glucose) and bottles were enclosed with air-tight stoppers to ensure anaerobic conditions throughout the experiment. At the beginning of simulation 0.1 % inoculums of the overnight grown *Cl. perfringens* culture was injected to TSGY-bottles. Test compounds or ethanol was added in 150 µl final volume from the respective stock solution according to the treatment. The simulation bottles were randomized to avoid artificial bias between treatments. The bottles were kept at even temperature of 37 °C and mixed 1 min before turbidity measurement at each time point. The total simulation time was 8h.

The turbidity was measured at the time points of 0.5, 3, 6 and 8 hours. The turbidity (optical density, OD) of growth media increases proportionally as *Cl. perfringens* cell number and cell density increases. Sometimes the highest concentrations of test compounds affect to the turbidity already in the beginning of simulation regardless of bacterial growth, and therefore the turbidity change of each separate simulation bottle is more informative in comparing the different test compounds or doses. Total gas production was measured at the end of the 8h simulation as an indicator of growth efficiency, since *Cl. perfringens* produces detectable amounts of gas due to the active metabolism during exponential growth.

Results

The results are illustrated in Figure 1a and 1b. Both untreated and digested TOFA treatments effectively inhibited the growth of *Cl. perfringens* almost completely still in the concentration of 0.001%, which was detected as lack of turbidity change in 8 hours (Figure 1a) and the production of less than 2 ml gas (Figure 1b).

The concentration 0.05% of TOFA, which is not shown in the figures, totally prevented the growth of *Cl. perfringens*.

The results show that TOFA resists gastrointestinal digestion and maintains its efficacy against the growth of *Cl. perfringens*. This result shows that the TOFA prevents or alleviates the onset of necrotic enteritis if given to broiler chicks or other species of poultry in the feed.

The experiment shows that the TOFA is very effective against the growth of *Cl. perfringens*, and that most of its activity can resist gastrointestinal digestion.

EXAMPLE 2.

Pathogen inhibition test

This experiment was conducted to compare the efficacy of TOFA with 8.5 % resin acids and competing products for their ability to inhibit the growth of pure cultures of three Gram-positive pathogens: *Clostridium perfringens, Staphylococcus aureus and Streptococcus suis in vitro*. The competing products were commercial natural plant extracts and a medium-chain fatty acid product. Plant extracts A-C are merchandised to have inhibitory effects against Gram+ pathogenic bacteria and they can be used e.g. in management of coccidiosis risk. Before the simulation, the bacteria were grown overnight as pure cultures in their specific growth medium. The bacterial cultures were used as inoculant in the experiment.

Products and product doses are presented in Table 1.

Product	Dose 1(kg/ton of feed)	Dose 2 (kg/ton of feed)
TOFA 8.5 % resin acids	0.25	0.50
Plant (Oregano) extract A	0.25	0.50
Plant extract B	0.25	0.50
Plant extract C	0.50	1.00
Medium chain fatty acid product (MCFA)	0.25	0.50

The test products were first weighed into the glass bottles. 15 ml of bacterial growth medium was added. Bottles for *Cl. perfringens* and *S. aureus* were prepared in anaerobic glove box, while the bottles for *S. suis* and *B. cereus* were prepared in aerobic environment. Next, the bottles were enclosed with airtight stoppers to ensure anaerobic conditions throughout the experiment for *Cl. perfringens* and *S. aureus*. A needle was pushed through the stoppers of the two aerobic bacteria to ensure oxygen supply for the cul-

ture. 150 ml of bacterial culture (see above) was added into each bottle to act as an inoculum (1% of the volume). The simulation time was calculated starting from the time of inoculating each vessel. During the simulation, the bottles were kept at 37°C temperature in a constant, slow shaking for eight hours. Optical density was measured at the time points of 0, 2, 4, 6 and 8 hours. The turbidity (optical density, OD) of growth media increases proportionally as bacterial cell density increases.

Each product was tested at two concentrations and three replicates per concentration. Dose 2 was the recommended dose of the commercial products. Each product concentration had also a control vessel into which microbes were not included (one replicate/product/dose). These treatments controlled for any potential increase in the cloudiness that the test products may have induced into the growth medium during the simulation time. The total number of simulation vessels was 123 per bacterial species.

Results

The results are illustrated in Figures 2a to 2f. The TOFA of the invention totally inhibited the growth of *Cl. perfringens* at both product levels at the 8-hour time point (Figure 2a and 2b).

S. aureus was not able to grow at all in the presence of TOFA at the studied concentrations, while the other products showed no inhibition at dose 1 (Figure 2c). Two of the other products showed partial inhibition at product dose 2 (Figure 2d).

TOFA fully prevented the growth of *Streptococcus suis* during the 8-hour simulation at both product doses (Figure 2e and 2f). At dose 2, MCFA product efficiently inhibited the growth of *S. suis* at the 8-hour time point (Figure 2f).

The experiment shows that the TOFA is much more effective against the growth of *Cl. perfringens*, *Staphylococcus aureus and Streptococcus suis* as the commercial plant extracts A-C claiming inhibitory effects against Gram+ pathogenic bacteria.

It is obvious to a person skilled in the art that, with the advancement of technology, the basic idea of the invention may be implemented in various ways. The invention and its embodiments are thus not limited to the examples described above; instead they may vary within the scope of the claims.

CLAIMS

1. A tall oil fatty acid for use in the prevention of growth of harmful bacteria in the animal digestive tract and/or in the prevention of intestinal disorders.

2. The tall oil fatty acid for use according to claim 1, characterized in that it comprises 1-10% (w/w) resin acids.

3. The tall oil fatty acid for use according to claim 1 or 2, characterized in that it comprises 2-9 % (w/w) resin acids.

4. The tall oil fatty acid for use according to any of preceding claims 1 - 3, characterized in that it comprises 5-9% (w/w) resin acids.

5. The tall oil fatty acid for use according to any of preceding claims 1 - 4, characterized in that it comprises 90-98% (w/w) fatty acids.

6. The tall oil fatty acid for use according to any of preceding claims 1 - 5, characterized in that it is dried.

7. A feed supplement, characterized in that it comprises the tall oil fatty acid for use as defined in claim 1.

8. The feed supplement according to claim 7, characterized in that it is effective in the prevention of growth of harmful bacteria and/or in the prevention of intestinal disorders.

9. The feed supplement according to claim 7 or 8, characterized in that the tall oil fatty acid comprises 1-10% (w/w), preferably 2-9 % (w/w), most preferably 5-9% (w/w) resin acids.

10. The feed supplement according to any of preceding claims 7 - 9, characterized in that the tall oil fatty acid is dried.

11. The feed supplement according to any of preceding claims 7 - 10, characterized in

that the tall oil fatty acid is absorbed into a carrier material.

12. A feed composition for use as defined in claim 1 comprising the feed supplement according to any of preceding claims 7 – 11.

13. A feed composition according to claim 12, characterized in that it comprises a feed supplement in an amount of 0.00001 – 0.5 % (w/w) of the dry weight of the total amount of feed.

14. A feed composition according to claim 12 or 13, characterized in that it comprises a feed supplement in an amount of 0.0005 – 0.1 % (w/w) of the dry weight of the total amount of feed.

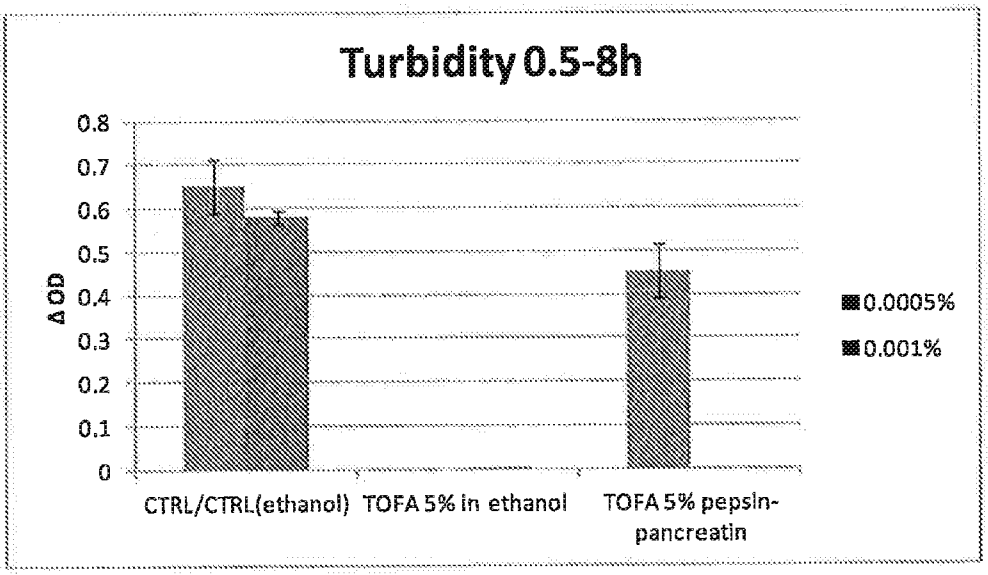

Turbidity 0.5-8h
ΔOD
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CTRL/CTRL(ethanol)
TOFA 5% in ethanol
TOFA 5% pepsin-pancreatin
0.0005%
0.001%

FIG 1a

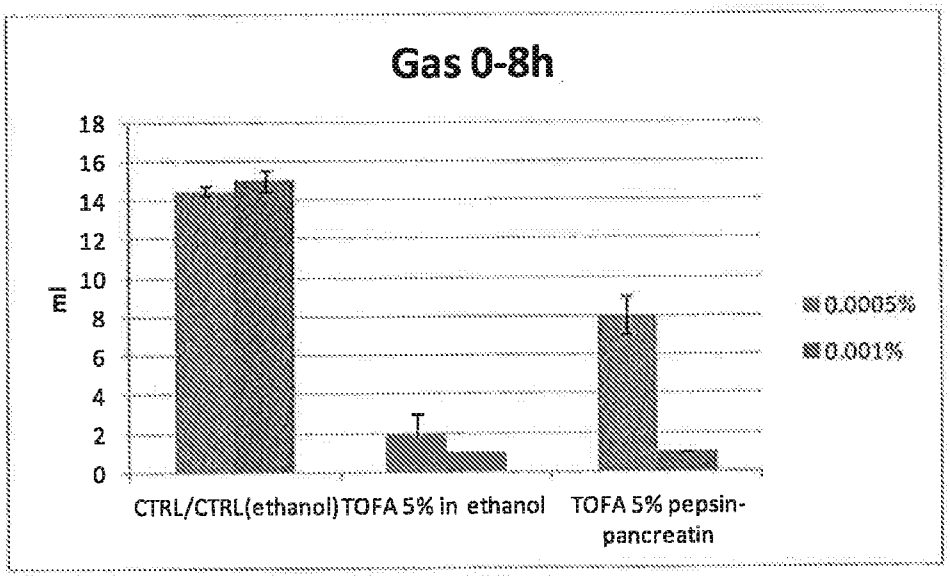

Gas 0-8h
ml
18
16
14
12
10
8
6
4
2
0
CTRL/CTRL(ethanol)
TOFA 5% in ethanol
TOFA 5% pepsin-pancreatin
0.0005%
0.001%

FIG 1b

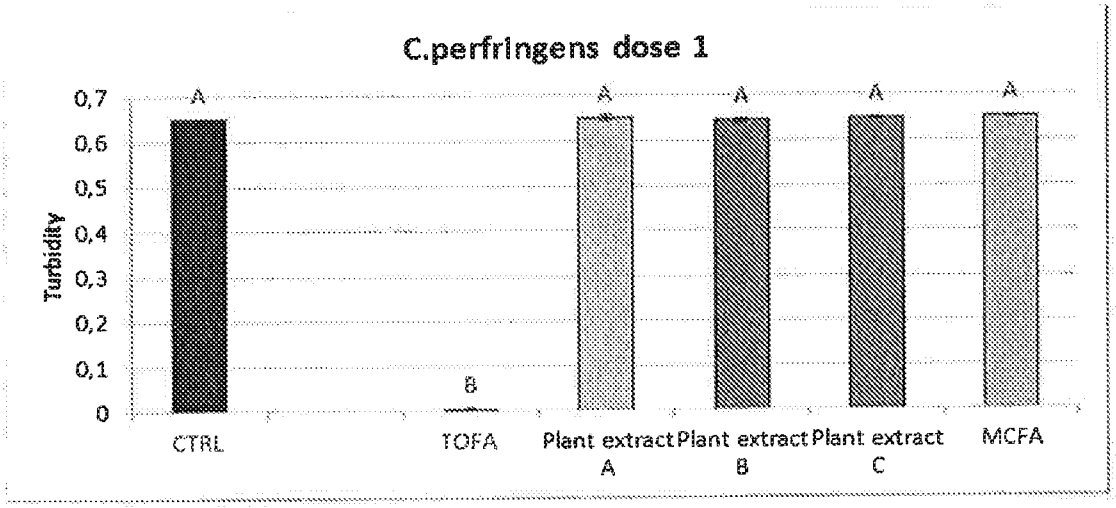


FIG 2a

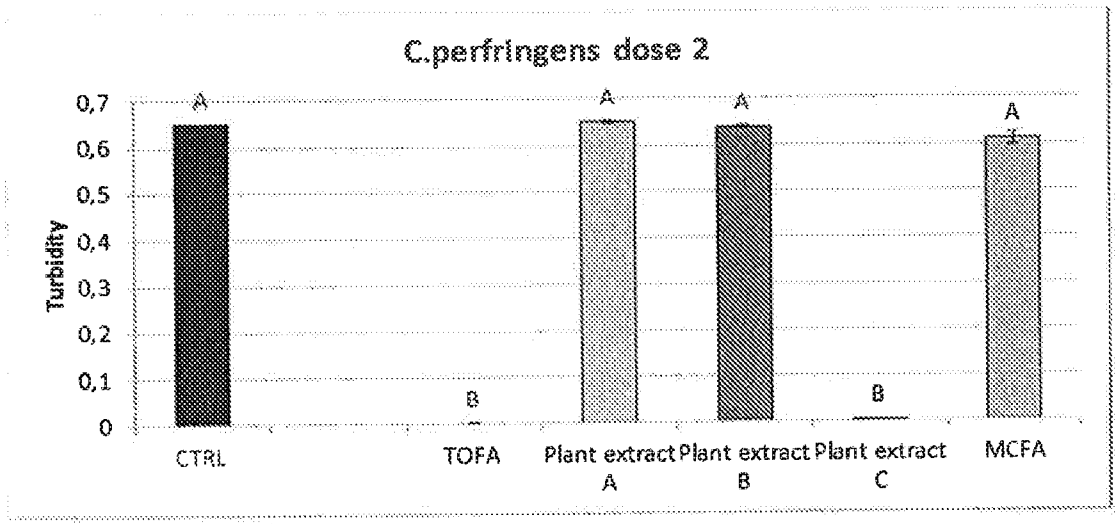


FIG 2b

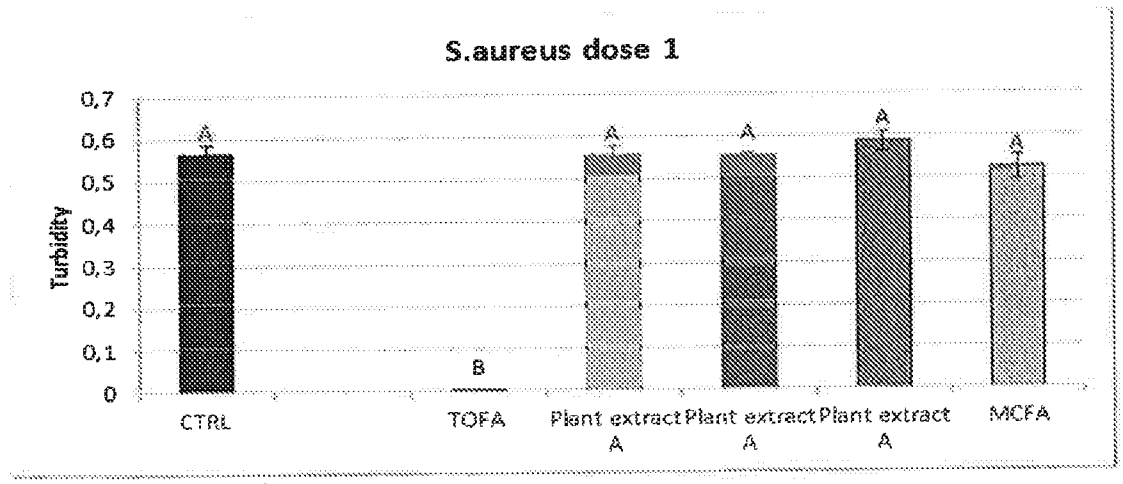


FIG 2c

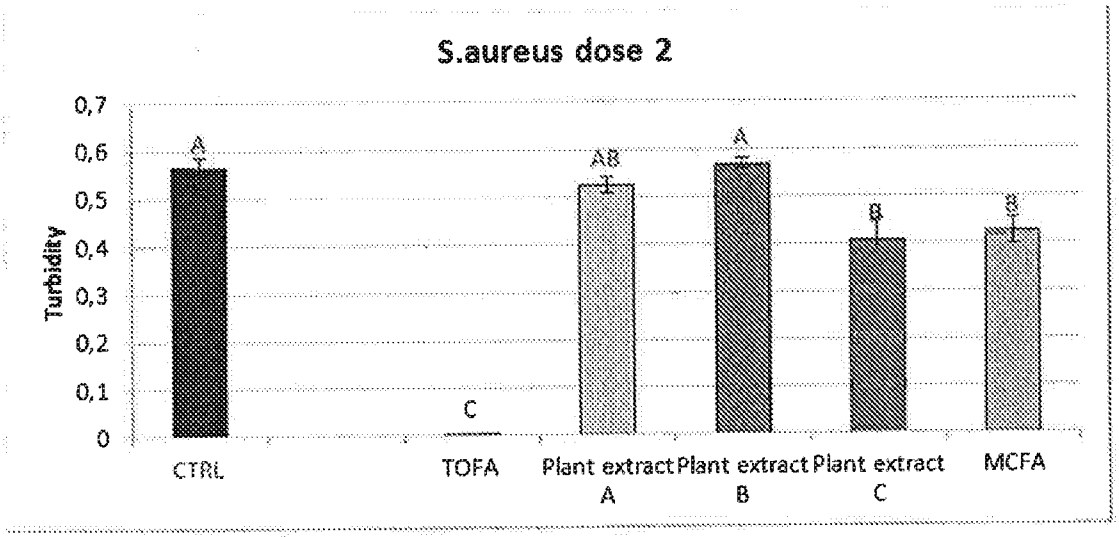


FIG 2d

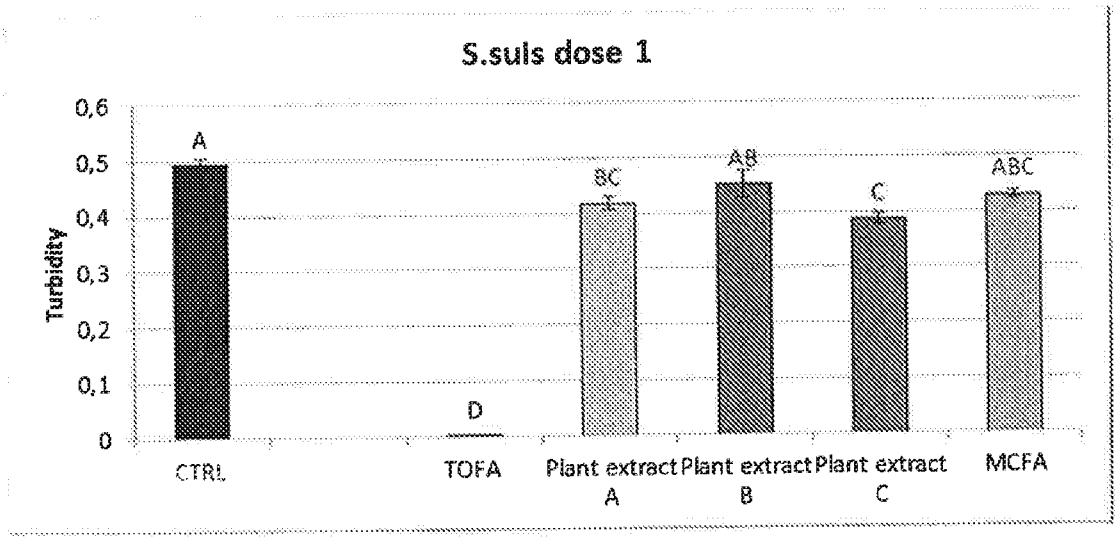


FIG 2e

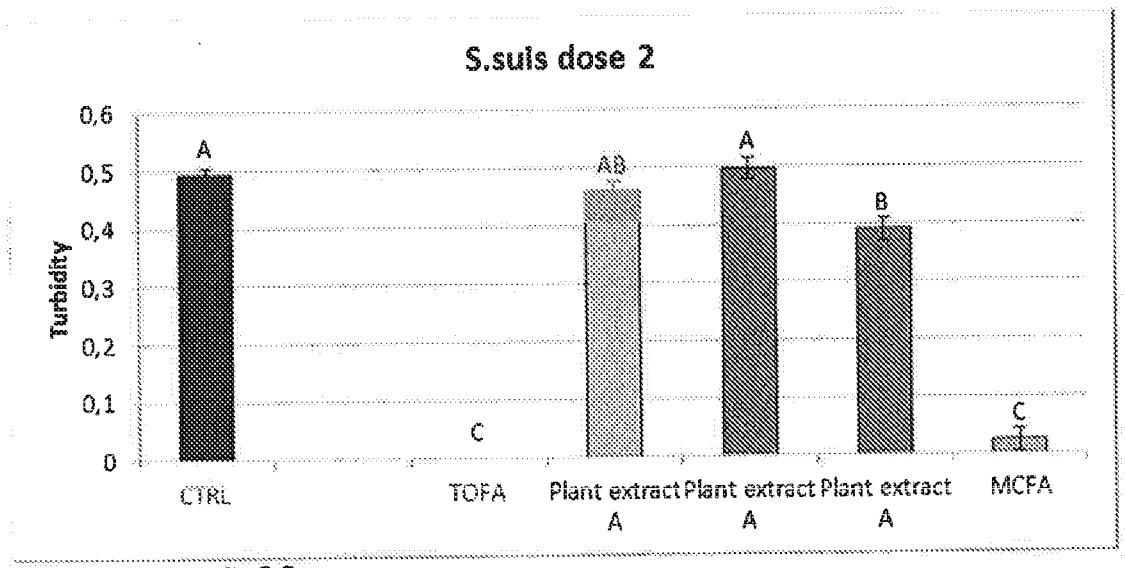


FIG 2f

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2014/050346

A. **CLASSIFICATION OF SUBJECT MATTER**

See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

FI, SE, NO, DK

Electronic data base consulted during the international search (name of data base, and, where practicable, search terms used)

EPO-Internal, WPI, AGRICOLA, BIOSIS, CABA, CHEMICAL ABSTRACTS, EMBASE, FROSTI, FSTA, MEDLINE, XPESP, XPESP2, XPOAC, XPRD, XPMISC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Product Data Sheet SYLFAT® 2LTC tall oil fatty acid [online], Arizona Chemical, [last modified 2009-07-20], [retrieved 2014-9-1] http://www.arizonachemical.com/Global/PDS/EU_product_data_sheets/SYLFAT%C2%AE%202LTC.pdf	1-14
A	WO 2008154522 A1 (ARIZONA CHEM [US]) 18 December 2008 (18.12.2008) abstract; paragraphs [0049] part r., [0054], [0055], [0057], [0070], [0072]; claims 1-6, 22	1-14
A	EP 0146738 A2 (AMERICAN CYANAMID CO [US]) 03 July 1985 (03.07.1985) abstract; page 2, line 19 – page 3, line 7; claims 1, 2	1-14
A	US 4443437 A (PROKOSCH WALTER G [US] et al.) 17 April 1984 (17.04.1984)	1-14

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" ddcument defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search	Date of mailing of the international search report
01 September 2014 (01.09.2014)	10 September 2014 (10.09.2014)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI2014/050346

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006286185 A1 (PROKOSCH WALTER G [US]) 21 December 2006 (21.12.2006) abstract; paragraphs [0006], [0012]; claims 1, 2	1-14
A	FI 41337 B (VALKE OSAKEYHTIOE [FI]) 30 June 1969 (30.06.1969) page 2, lines 8-18; page 3, Example 1; pages 4-5, Example 3	1-14

INTERNATIONAL SEARCH REPORT
Information on Patent Family Members

International application No.
PCT/FI2014/050346

Patent document cited in search report	Publication date	Patent family members(s)	Publication date
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