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(54) Title: A METHOD OF PREVENTING ANIMALS FROM DISEASES AND IMPROVING IMMUNE FUNCTION OF ANIMALS

(54) 发明名称: 一种预防动物疾病并促进动物生长发育的方法

(57) Abstract

A method of promoting growth and improving immune function of animals is disclosed in the present invention, in which bioactive agent(s) extracted from seed dregs of oil-bearing crops, especially triterpene type saponin(s) extracted from seed dregs of oil-tea camellia or tea, are used as feed additives.

(57) 摘要

本发明提供了一种促进动物生长，提高动物免疫机能的方法，它包括给动物服用一种得自油料作物种子残渣的具有生物活性的添加剂，特别是由油茶饼或茶叶籽饼中提取的三萜类皂素。

以下内容仅供参考

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Abstract

A method of promoting growth and improving imumune function of animals is described in the present invention, in which a bioactive agent extracted from seed cake is used as feed additive.

Method of Preventing Animal Diseases and Promoting Animal Growth and Development

Technical Field

The present invention relates to a method of preventing animal diseases and promoting animal growth and development. In particular, the method of the present invention comprises extracting bioactive ingredients from oil plants and adding them into feed and food as feed additive and nutrient, respectively, to improve the immune function of animals and to promote the growth and developement of animals.

Background Art

Plants belonging to Camellia L. are very popular in China, Japan, India and many other South-East Asian countries. Among of them, plants from three species C. sinensis, C. oleifera and C. japonica, are of significant economical value. For example, Leaves of C. sinensis plants can be used to produce tea, seeds of C. oleifera plants can be used to produce edible oil, C. japonica plants are also useful as ornamental plants.

With the development of modern feed industry, a lot of antibiotics are used as feed additive to prevent animal diseases and to promote animal growth. In 1990, about 2000 tons of antibiotics were used as feed additives in China, about 1350 tons and 1300 tons of antibiotics were used in West Europe and Japan, respectively, in poultry and livestock feed. Recently, sale value of antibiotics reached to US $1.019 billions in USA and utilization of antibiotics is increasing by 3% annually world

wide. Long-term utilization of antibiotics, however, can result in drug-resistance of microbes which will create world-wide environment pollution via R-factor translocation, and residue problem in animal products which will be a threaten to human health. WHO (World Health Organization) and FAO (Food and Agriculture Organization, United Nations) are taking great consideration about the research on alternatives of antibiotics as feed additives. Currently, research and development on natural material as feed additive made a lots of progress. For example, feed microbes and traditional Chinese medicines had been used extensively. However, feed microbes are prone to be inactive during feed processing and can only replace antibiotics partially. Traditional Chinese medicine additives can not be used extensively, due to their sophistication in composition and instability in function. Therefore, there is necessity to use natural bioactive ingredients as feed additive to replace antibiotics, exclude antibiotics residue in animal products, improve product quality, and alleviate environment pollution.

Aoyama S. first separated thea saponin from tea seed cake in 1931. ***(Journal of Pharmacology, 51(5): 367, 1931),*** however he did not obtained this pure chemical. In 1952, Ishidate M. and Ueda Y. of Tokyo University obtained the pure crystals of thea saponin (Ishidate M. and Ueda Y., ***Journal of Pharmacology, 72 (11): 1525, 1952.***). From 1970's, series of studies on separation, characterization and utilization of thea saponin were conducted in major tea-producing countries. Many extracting methods and products had been developed during this period.

It is now clear that saponin obtained from Camellia L. belongs to triterpenoid compounds. It is a group of complicated compounds composed of aglycone ($C_{30}H_{50}O_6$), sugars and organic acids. The chemical structures of saponin obtained from Camellia L. have been disclosed in many references, for example, in Yoshioka, I. et al., Chemical Pharmacology Bulletin (Tokyo) 1970, 18. 1610; Yoshioka, I. et al.,

Thea sapogenol A., The major sapogenol of the seeds saponin of thea Sinensis L., Tetrahedron Letters, 1966 (48): 5979-5984, 5973-5978; and Yoshioka, I. et al., Saponin and Sapogenol III seeds sapogenols of Thea Sinensis L (3) , Thea sapogenol E and minor sapogenols, Chem. Phar. Bull. 1971, 19 (6) 1186-1199.

Development in thea saponin research offers great opportunity for complete utilization of by-products of tea seed and tea leaves. In 1972, Roberts G.R and his colleague (Roberts G.R. et al., ***Tea QUARTERLY 43 (3), 1972***) from Srilanka improved thea saponin extracting technology, industrialized its preparation, and suggested several other methods to use tea seed cake from Camellia plants (Wickremastinghe R.L. et al., 1972. ***Tea Quarterly 43 (3)***). As estimated by Yaziciglu T. and his colleague, about 600 tons of thea saponin could be extracted from 15,000 tons of tea seed in Turkey. So far, industrialization of thea saponin production has been possible, and Nippon Isome Grease Chemical Co. already commercialized thea saponin.

Saponin obtained from Camellia L. has extensive utilization in industry. Its utilization in medicine was the earliest research field, however, this field developed slowly though there are so many pharmacologist. Pharmacological effects of the saponin include antiosmosis, antiphlogistics, and control of coughing. It was reported that the saponin had special effect on many kinds of oedema (dropsy).

Industry utilization of saponin obtained from Camellia L. is a newly developed field. It could be used to produce kinds of water and oil emulsion, preservative, foaming agent in beer industry and detergent in daily industry. It could maintain the color of the fabrics as it had no damage to dye on the fabrics. When used in laundry, it prevents shrinking of woolen products and maintains the luster of the fabrics as well. The saponin can also be used in photograph industry.

Saponin obtained from Camellia has extensive usage in agriculture, expecially as insecticide, germicide and binding agent in spray pesticide. Its major benefit is to avoid pesticide residue and protect environment.

However, research on utilization of saponin obtained from Camellia as feed additive to replace antibiotics had not been documented yet, nor the effects of said saponin on animal growth and immune function promotion.

After research and development of saponin obtained from Camellia L. for many years, the present inventor has unexpectedly found that saponins extracted from seed cake of Camellia L. could improve the immune function and had the effects of anti-bacteria and anti-viruses. It is safe under some conditions and can enhance the growth of the animals.

In accordance with one aspect of the invention there is provided an animal feed composition comprising: (a) an animal feed; and (b) triterpenoid saponin obtained from Camellia L. plants.

In accordance with a further aspect of the present invention there is provided a method of improving immune function and enhancing anti-bacterium and anti-viruse activities of human and animals, which comprises administering a composition comprising triterpenoid saponin obtained from Camellia L. plants to human and animals.

In accordance with a another aspect of the present invention there is provided a method for enhancing animal performance and for improving meat quality, which comprises feeding animals with an animal feed composition according to the present invention.

Detailed Description of the Invention

As used in the context of the present application, the term "seed cake" refers to residues obtained from seeds of oil-bearing plants after extraction of oil therefrom.

The term "Camellia seed cake" refers to seed cake obtained from Camellia L. plants, such as from C. sinensis, C. oleifera and C. japonica plants.

Triterpenoid saponin used in this invention was extracted from oil plants. Preferably, it is obtained from plants belonging to camellia family, and most preferably it is obtained from leaves and seeds of Camellia plants.

The process used in the present invention for the production of triterpenoid saponin is described as follows.

Seed cake after oil extraction is grounded, soaked in alcohol and other organic reagents. Organic extract is filtered, condensed and dried to obtain triterpenoid saponin powder. Temperature ranges from 20 to 50 ℃, preferably from 30 to 40 ℃. Concentration of the organic reagents rangents from 60 to 90%. Drying method can be vacunm-drying or sprap-drying.

Saponin obtained according to this invention can be used directly as feed additive or health care agent. It can also be used in combination with trace elements.

Dosage and application method of this saponin preparation are similar to conventional feed additive and health care agents. Its content in feed can be in the

range of 50-1500 ppm, preferably in the range of 250-750 ppm.

This invention offers a bioactive ingredient from natural Camellia seed cake to be used as feed additive. It can replace antibiotics in conventional feed completely, thus avoid antibiotic residues in animal products, produce high-quality animal products, reduce environment pollution and increase animal production performances. It can also be used as nutritive health care agent. Therefore, low valued oil-seed meal can be used effectively and have a better social and economical benefit.

The following examples are used to illustrate the invention in more detail, but they are not intended to limit the scope of the present invention in any way.

Example 1

Preparation of bioactive feed additive A

8000 grams of Camellia olefera seed cake were extracted with 83 percent alcohol (v/v) for three times. All the organic extracts were combined and vacuum-dried. 800 grams of extracted powder were obtained, which used directly as feed additive.

Example 2

Preparation of bioactive feed additive B

25 grams of zinc sulfate of feed grade and 25 grams of manganese sulfate of feed grade were added to 1000 grams of the feed additive A obtained in Example 1. After mixing evenly, feed additive B was then obtained.

Example 3

Preparation of bioactive feed additive C

100 grams of zinc sulfate of feed grade, 100 grams of manganese sulfate of feed grade and 50 grams of vitamin C were added to 1000 grams of the above mentioned additive A . After mixing evenly, feed additive C was then obtained.

Example 4

Improvment of survival rate of livestock

From July 1992 to June, 1994, 3108 one-day old chicken (4 batches), 320 eleven-day old piglets (8 batches) and 600 growing chicken for egg purpose were allocated into control and test groups to test survival rates. Feed used in control groups were AMV broiler complete feed, PCS pig complete feed and Wanghai brand complete feed for chicken layers. The formulations of the three feed compositions were as follows:

Formulation of PCS Complete Feed for Pig

Ingredients	piglet period	growing period
corn (%)	51.7	48.2
wheat bran (%)	16.00	15.0
middlings (%)	10.0	20.0
fish meal (%)	6.0	4.0
limestone (%)	1.0	1.0
$CaHPO_3$ (%)	0.5	0.5
NaCl (%)	0.3	0.3
additives (%)	1.0	1.0
Olaquindox (ppm)	80.0	60.0

AMV Complete feed for broiler

Ingredients	0-21 days	22-49 days
corn (%)	57	67
soybean meal (%)	30	23
middilings (%)	5	-
concentrate 1 (%)	5	-
concentrate 2 (%)	-	5
fishmeal (%)	3.0	2.0
yeast (%)	-	3.0
colistin sulfate (ppm)	6.0	6.0
bacitracin zinc (ppm)	30.0	30.0
coccidiostatics (ppm)	-	125 (22-42 days)

Wanghai brand feed for growing layers

corn %	60
wheat bran (%)	10.0
soybean meal (%)	17.0
fishmeal (%)	9.0
bone meal (%)	2.0
additive (%)	2.0
terramycin (ppm)	100

In test groups, 500 ppm of bioactive additive obtained from practical example 1 was used to replace all the antibiotics in the control diets. The survival rates after 49, 120 and 21 days of experiments were estimated for broilers, piglets and growing layers respectively. The results were listed in Table 1.

Table 1. Survival rates of livestock

group	broiler		growing layers		piglet	
	tested	survival rate (%)	tested	survival rate (%)	tested	survival rate(%)
saponin	1845	95.30	300	88.00**	160	100.00
antibiotics	1263	94.4	300	77.00	160	98.13

** P<0.01

Results indicated that Sasanqua saponin could be used to replace antibiotics in the diets. The survival rates were increased by 0.95%, 14.29% (P<0.01) and 1.91% respectively for broilers, growing layers and piglets.

Example 5

Effect of increasing live weight gain of livestock

Under the same experimental designs as in example 4, the effects of the bioactive agents obtained by this invention on body weight gain were tested at day 49 (Table 2). The results were showed in table 2 below.

Table 2 Body weight gain of livestock

group	broiler			piglet		
	tested	net gain (g/each)	ADG (g/day)	tested	net gain (kg/each)	ADG (g/day)
saponin	1845	2019.8	41.2	160	25.17	508.27
antibiotics	1263	1971.2	40.2	160	22.92	463.60

In broilers, net gain increased by 48.6 grams/bird and ADG increased by 2.49%. In piglet, net gain increased by 2.25 kg/animal and ADG increased by 9.64%.

Example 6

Effect of increasing feed efficiency

Under the same experimental conditions as described in example 4, the effects of the feed additive of the present invention on feed efficiency were tested. The results were showed in table 3.

Table 3 Feed efficiency

group	broiler		piglet	
	tested	feed:gain	tested	feed:gain
saponin	1845	2.06:1**	160	2.42:1
antibiotics	1263	2.26:1	160	2.54:1

**: $p<0.05$

Results suggested that replacement of antibiotics with bioactive saponin decreased ratio of feed to gain by 9.71% ($P<0.05$) and 4.72% respectively for broilers and piglets.

Example 7

Effect of improving meat nutrient contents

Under the same experimental design as in example 4, fat and amino acid profiles in chicken at day 49 were determined. Results suggested that saponin could improve chicken nutrient quality, especially threonine content by8.38 % ($P<0.05$).

Example 8

Effect of improving processing value of livestock products

Under the same experimental design as in example 4, some processing indices

at day 49 were tested. Saponin replacement increased dressing percentage and total meat pigment by 1.93 % and 7.48 % respectively in broilers. Meat water lose percentage and pH decreased by 1.37 % and 2.50 % respectively. Results indicated that saponin addition is beneficial to meat processing and storage.

Example 9

Effect of reducing heavy metals in meat

Under the same experimental design as in example 4, contents of heavy metal elements in meat were determined with atomic absorption spectrometer at day 49. Result indicated that saponin reduced Cd and Pb contents in meat by 94.41 and 38.28 % respectively in broilers.

Example 10

Effect of antioxidation

To conventional feed, 750 ppm bioactive agent obtained in example 1 was added. After incubation for 49 days at 40 ℃, acidity of the feed was determined by KOH titration method, peroxidation value was determined by sodium thiosulfate method, and the content of Vitamin A was determined by HPLC. Results suggested that acidity and peroxidation value were reduced by 38.79 % and 21.28 % ($P<0.05$) respectively, and saponin had protection over fat and Vitamin A.

Example 11

Effect of increasing immune function and antiviruses

Saponin obtained from example 1 was added into broiler feed at a dosage of 750 ppm. After IBD viruses were injected into chickens. at day 28 , blood and

spleen samples were taken at day 34. As a result, Immunoglobin, T lymphocytes transformation rate, interleukin 2(IL-2) and erythrocyte C3b receptors were increased by 11.56 % ($P<0.01$), 54.09 % ($P<0.05$), 52.66 % ($P<0.05$) and 21.71 % ($P<0.05$) respectively. This suggested that saponin could improve immune function and has the effect of antiviruses.

Example 12

Effect of free radical scavenge and antimutation

Light emision analysis results showed that saponin obtained from exmaple 1 cleared about 94.40% of superoxide anion radical (O_2^-) ($P<0.01$) and 78.19 %($P<0.01$) hydroxyl radical (OH^-). In adult cocks and hens, ethyl methanesulfonate (EMS) was used at the dosage of 82 mg/kg and 80 mg/kg respectively via intramuscular injection for three days. Sasanqua saponin addtion at 750 ppm decreased cock sperm deformation rate by 58.96 % ($P<0.01$), increased hen ovulation rate by 28.64 %, and increased egg fertility by 75.54 ($P<0.01$) . These results suggested that the extracted bioactive additive was effective in protecting cells against deformation induced by alkylating agents.

Example 13

Effect of anti-bacteria

750 ppm extracted bioactive agent was added into culture dish to evaluate its antibacterium effect. Results indicated that suppressing concentrations for ***E. Coli*** and ***salmonella*** were 1.25-0.5 mg/mL and 0.1563-0.0390 mg/mL respectively.

Example 14

Effect of increasing hormone, protein and enzyme levels

Under the same experimental design as in example 4, venous blood samples were taken at day 49. Results showed that saponin addition increased serum testosterone level by 25.61% ($P<0.05$), serum total protein content by 11.08%, alkaline phosphatase activity by 19.023%, amylase activity by 16.79 % and total proteolytic enzyme activity by 49.37%.

Example 15

Effect on growth of aquatics

Addition of 10 ppm Sasanqua saponin in turtle, prawn and eel diets improved their survival rates and growth rates.

Example 16

Safety test of Sasanquasaponin

Tea saponin obtained from example 1 was added at 5000 ppm in the diet of rat. After 30 days of feeding, no abnormal signs were observed in rat somatic cells and reproductive cells. Test results indicated that tea saponin is safe as feed additive.

It will be appreciated that it is not intended to limit the invention to the above examples only, many variations thereto and modifications thereof being possible to one skilled in the art without departing from its scope, which is defined by the appended claims.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers or steps but not the exclusion of any other integer or group of integers or steps.

The claims defining the invention are as follows:

1. An animal feed composition comprising:

(a) an animal feed; and

(b) triterpenoid saponin obtained from Camellia L. plants.

2. The composition according to Claim 1, wherein the content of component (b) is about 50-1500 ppm by weight of feed.

3. The composition according to Claim 2, wherein the content of component (b) is about 250-750 ppm by weight of feed.

4. A composition according to Claim 1, wherein the animal includes livestock and poultry.

5. A composition according to Claim 4, wherein the livestock and poultry are chicken, duck or pig.

6. A composition according to Claim 1, wherein said Camellia L. plants are selected from C. sinensis, C. oleifera and C. japonica.

7. A composition according to Claim 6, wherein the triterpenoid saponin is obtained form the seeds or leaves of said plants.

8. A composition according to Claim 7, wherein the triterpenoid saponin is extracted from Camellia seed cake with an organic solvent at room temperature.

9. A method of improving immune function and enhancing anti-bacterium and anti-viruse activities of human and animals, which comprises administering a composition comprising triterpenoid saponin obtained from Camellia L. plants to human and animals.

10. A method for enhancing animal performance and for improving meat quality, which comprises feeding animals with an animal feed composition according to claim 1.

11. Animal feed compositions according to anyone of claims 1-8 or uses thereof, substantially as hereinbefore described with reference to the Examples.

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