Title: METHOD FOR INCREASING THE SPEED OF RECOVERY AND/OR THE SPEED OF WOUND HEALING OF INJURED ANIMALS

Abstract: This invention is in the field of animal food, in particular food for wounded animals. Surprisingly, it has now been found that the speed of recovery of wounded animals may be increased by feeding the animal with a certain amount of natural occurring immune enhancing ingredients such as beta-glucans and/or phytohormones like auxin or gibberelic acid. Also, combinations of these substances, in particular the combination of beta-glucans and phytohormones, were shown to have a synergistic effect in that they improved the recovery speed of the animal more than the individual components on their own.
METHOD FOR INCREASING THE SPEED OF RECOVERY AND/OR THE SPEED OF
WOUND HEALING OF INJURED ANIMALS

This invention is in the field of animal food, in particular food for
wounded animals. Food according to the invention increases the speed of wound
healing of injured animals.

Wound healing is the process of repair that follows injury to the skin
and other soft tissues. Symptoms of wound healing include swelling, stiffness,
tenderness, discoloration, skin tightness, scabbing, itching, and scar formation.

Wounds may result from trauma or from a surgical incision. Wounds
may also result from bone fracture or from giving birth. In addition, pressure ulcers (also
known as decubitus ulcers or bed sores), a type of skin ulcer, might also be considered
wounds. The capacity of a wound to heal depends in part on its depth, as well as on the
overall health and nutritional status of the individual.

Following injury, an inflammatory response occurs and the cells below
the dermis (the deepest skin layer) begin to increase collagen (connective tissue)
production. Later, the epithelial tissue (the outer skin layer) is regenerated. Dietary
modifications and nutritional and herbal supplements may improve the quality of wound
healing by influencing these reparative processes or by limiting the damaging effects of
inflammation.

Over the counter topical antibiotics, such as neosporin (Myciguent®),
bacitracin (Baciguent®), and combinations of the two with polymyxin B (Neosporin®,
Polysporin®) are used to treat skin infections and promote wound healing. Prescription
strength topical antibiotics, such as metronidazole (MetroGel®) and mupirocin
(Bactroban®), might be necessary to treat infection and promote healing.

Other treatment includes keeping the wound clean, dry, and covered.
Surgical treatments, such as stitches and removal of damaged tissue, may be
recommended.

Building and repairing tissue requires adequate amounts of calories
and protein to fuel the repair mechanisms, as the skin and underlying tissues are made
of protein. While major wounds from extensive injuries or major surgery significantly
raise protein and calorie requirements, optimal healing of minor wounds should not
require changes from a typical, healthful diet.¹ In a study of malnourished people with
skin ulcers, those who were given a diet containing 24% protein showed a significant
reduction in the size of the ulcer, whereas those given a diet containing 14% protein had no significant improvement. This study suggests an increase in dietary protein can improve wound healing in malnourished people. It is not known whether the same benefit would be observed in well-nourished people.

Supplementation with bromelain, an enzyme derived from pineapple stem, prior to and following a surgical procedure has been shown to reduce swelling, bruising, healing time, and pain. Bromelain supplementation has also been shown to accelerate the healing of soft-tissue injuries in male boxers. The amount of bromelain used in these studies was 40 mg four times per day, in the form of enteric-coated tablets. Enteric-coating prevents the stomach acid from partially destroying the bromelain. Most currently available bromelain products are not enteric-coated, and it is not known if such products would be as effective as enteric-coated bromelain.

Thiamine (vitamin B1), pantothenic acid (vitamin B5), and other B vitamins have all been shown to play a role in wound healing in animal studies. For this reason, although human research is lacking, some alternative healthcare practitioners recommend a high-potency B vitamin supplement to promote wound healing.

Vitamin C is needed to make collagen (connective tissue) that strengthens skin, muscles, and blood vessels and to ensure proper wound healing. Severe injury appears to increase vitamin C requirements, and vitamin C deficiency causes delayed healing. Preliminary human studies suggest that vitamin C supplementation in non-deficient people can speed healing of various types of wounds and trauma, including surgery, minor injuries, herniated inter-vertebral discs, and skin ulcers. A combination of 1–3 grams per day of vitamin C and 200–900 mg per day of pantothenic acid has produced minor improvements in the strength of healing skin tissue.

Zinc is a component of many enzymes, including some that are needed to repair wounds. Even a mild deficiency of zinc can interfere with optimal recovery from everyday tissue damage, as well as from more serious trauma. One controlled trial found the healing time of a surgical wound was reduced by 43% with oral supplementation of 50 mg of zinc three times per day, in the form of zinc sulfate.

Preliminary and controlled studies of people with severe burns and other types of injuries showed that supplementation with 10–30 grams of ornithine alpha-ketoglutarate (OKG) per day significantly improved wound healing and
decreased the length of hospital stays. Improved healing from major trauma and surgery has also been demonstrated with oral supplements including several grams per day of glutamine.23

Vitamin A plays a central role in wound healing.24 but the effect of supplemental vitamin A in people who have suffered a minor injury and are not vitamin A-deficient remains unclear. Vitamin A supplements have been shown to improve healing in animal studies,25 and may be especially useful in a topical ointment for skin injuries in people taking corticosteroid medications.26 Although there are no studies in humans, some doctors recommend 25,000 IU of vitamin A per day, beginning two weeks prior to surgery and continuing for four weeks after surgery.

Animal studies have shown that supplementing with vitamin E can decrease the formation of unwanted adhesions following a surgical wound. In addition, wound healing was more rapid in animals fed a vitamin E-rich diet than in those fed a standard diet.27 In another study, however, wound healing was inhibited by supplementation with a massive amount of vitamin E (equivalent to about 35,000 IU).28 This adverse effect of vitamin E was prevented by supplementation with vitamin A. Although the relevance of these studies to humans is not clear, many doctors recommend supplementing with both vitamins A and E in order to enhance wound healing and prevent adhesion formation. Typical amounts recommended are 25,000 IU of vitamin A per day and 400 IU of vitamin E per day, beginning two weeks prior to surgery and continuing for four weeks after surgery.

Topical application of vitamin E is sometimes recommended for preventing or treating post-injury scars, although only three controlled studies have been reported. Two of these trials found no effect on scar prevention after surgery,29 30 and one trial found vitamin E improved the effect of silicon bandages on large scars called keloids.31

Copper is a required cofactor for the enzyme lysyl oxidase, which plays a role in the cross-linking (and strengthening) of connective tissue.32 Doctors often recommend a copper supplement as part of a comprehensive nutritional program to promote wound healing. A typical amount recommended is 2–4 mg per day, beginning two weeks prior to surgery and continuing for four weeks after surgery.

Other trace minerals, such as manganese, copper, and silicon, are known to be important in the biochemistry of tissue healing.33 34 35 36 However, there
have been no controlled trials exploring the effect of oral supplementation of these minerals on the rate of healing.

Glucosamine sulfate and chondroitin sulfate may both play a role in wound healing by providing the raw material needed by the body to manufacture connective tissue found in skin, tendons, ligaments, and joints.\textsuperscript{37} Test tube and animal studies have found that these substances, and others like them, can promote improved tissue healing.\textsuperscript{38 39 40 41 42} One controlled trial in humans found that wounds healed with greater strength when they were treated topically with a chondroitin sulfate-containing powder.\textsuperscript{43} However, no research has investigated the value of oral supplements of glucosamine or chondroitin for wound healing in humans.

Arginine supplementation increases protein synthesis and improves wound healing in animals.\textsuperscript{44} Two trials have shown increased tissue synthesis in surgical wounds in people given 17–25 grams of oral arginine per day.\textsuperscript{45 46}

Carnosine is a small molecule composed of the amino acids histidine and alanine. The exact biological role of carnosine is not completely understood, but animal research demonstrates that it promotes wound healing.\textsuperscript{47}

While many herbs may be useful in wound healing, it is important that wounds be properly cleaned and dressed before any herbal preparations are applied. This will prevent infection.

In animal studies of skin inflammation, both topical and oral aloe vera have proven beneficial in decreasing inflammation and promoting cellular repair.\textsuperscript{48 49} Topical aloe vera has facilitated wound healing in controlled human research, as well.\textsuperscript{50} In one controlled trial, however, topical aloe vera gel was inferior to conventional management of surgical wounds.\textsuperscript{51}

One preliminary trial found that a gotu kola extract helped heal infected wounds (unless they had reached bone).\textsuperscript{52} A review of French studies suggests that topical gotu kola can help wounds.\textsuperscript{53} One study found gotu kola extract helpful for preventing and treating enlarged scars (keloids).\textsuperscript{54} Standardized extracts of gotu kola containing up to 100% total triterpenoids are generally taken, providing 60 mg once or twice per day. Animal studies have shown that constituents in gotu kola, called asiaticosides, increase antioxidant levels during wound healing and facilitate repair of connective tissues.\textsuperscript{55 56}
Horse chestnut contains a compound called aescin that acts as an anti-inflammatory and reduces edema (swelling with fluid) following trauma, particularly sports injuries, surgery, and head injury.\textsuperscript{57} A topical aescin preparation is popular in Europe for the treatment of acute sprains during sporting events.

A topical preparation of chamomile combined with corticosteroids and antihistamines has been used to speed wound healing in elderly people with stasis ulcers caused by inadequate circulation,\textsuperscript{58} as well as in people who had tattoos removed.\textsuperscript{59} Topical use of chamomile ointment was also found to successfully treat mild stasis ulcers in elderly bedridden patients.\textsuperscript{60}

Topical application of honey has been used since antiquity to accelerate skin wound healing.\textsuperscript{61} Honey has been shown to inhibit the growth of several organisms responsible for wound infections.\textsuperscript{62} 63 64 In one preliminary study, nine infants with large, open infected wounds that failed to heal with conventional treatment were treated successfully with topical application of honey.\textsuperscript{65} Fresh unprocessed honey was applied to wounds in amounts of 5–10 ml twice daily for a period of 21 days. All infants showed marked clinical improvement after 5 days, and the wounds were closed and free of infection by 21 days. The use of honey to treat wounds should be supervised by a doctor.

Used topically, some practitioners consider arnica to be among the best vulnerary (wound-healing) herbs available.\textsuperscript{66} Topical use of arnica is approved by the German government for improving wound healing.\textsuperscript{67} Arnica is poisonous if taken internally.

Calendula flowers were historically considered beneficial for wound healing, reducing inflammation and fighting infection as a natural antiseptic.\textsuperscript{68} Like echinacea, calendula is approved in Germany for use in treating poorly healing wounds.\textsuperscript{69} Generally 1 tablespoon (15 grams) of calendula flowers is steeped in hot water for 15 minutes, then cloths are dipped into the liquid to make compresses. Such compresses should be applied for at least 15 minutes, initially several times per day, then tapering off as the wound improves.

Traditional herbalists sometimes recommend the topical use of herbs such as St. John's wort, calendula, chamomile, and plantain, either alone or in combination, to speed wound healing. Clinical trial in humans have not yet validated this traditional practice.
Echinacea is used among European practitioners of herbal medicine to promote wound healing\textsuperscript{70} and is approved by the German government for this use.\textsuperscript{71} Creams or ointments are applied several times a day to minor wounds.

Comfrey has anti-inflammatory properties that may decrease bruising when the herb is applied topically.\textsuperscript{72} Comfrey is also widely used in traditional medicine as a topical application to help heal wounds.\textsuperscript{73} Witch hazel can also be used topically to decrease inflammation and to stop bleeding.\textsuperscript{74} Native Americans used poultices of witch hazel leaves and bark to treat wounds, insect bites, and ulcers.\textsuperscript{75} Horsetail can be used both internally and topically to decrease inflammation and promote wound healing.\textsuperscript{76}

Despite all these products that may improve the speed of wound healing there is still room for alternative products that can play a role in this beneficial process.

Surprisingly, it has now been found that the speed of recovery and/or the speed of wound healing of injured animals may be increased by feeding the animal with a certain amount of natural occurring immune enhancing ingredients such as beta-glucans and/or phytohormones like auxin or gibberellic acid. Also, combinations of these substances, in particular the combination of beta-glucans and phytohormones, more in particular the combination of beta-glucans and free IAA were shown to have a synergistic effect in that they improved the speed of recovery of the animal and/or the speed of wound healing more than these individual components on their own.

The term wounded animals or injured animals is used herein to refer for instance to animals that have experienced a physical trauma, such as bone fractures, flesh wounds, internal wounds or have recently given birth or have undergone surgery. Infection with microorganisms such as bacteria and parasites may also cause wounds that can be healed better with a preparation according to the present invention.

Phytohormones are herein defined as molecules that function to coordinate plant growth and development. The compounds that have been considered as plant hormones are for instance: indole-3-acetic acid (auxin), cytokinin, gibberellin, gibberellic acid, ethylene, abscisic acid. In addition, brassinosteroids, jasmonic acid and salicylic acid have been shown to have important growth regulating activities and are considered to function as Phytohormones.

Particularly good results were obtained when the animal feed either alone or in combination with beta-glucans was supplemented with free IAA instead of conjugated IAA. The term “free IAA” is used herein to indicate that the free IAA is in the
free or acid form, whereas the term “conjugated IAA” refers to IAA that is conjugated via ester linkages or via amide linkages.

Free IAA and conjugated IAA are known compounds. Free IAA is a naturally-occurring plant growth phytohormone which has been extensively studied. In plants, most of the IAA occurs in a conjugated form (Slovin et al. 1999, Biochemistry and molecular biology of plant hormones, Elsevier, Amsterdam. P115-140), either conjugated to sugars via ester linkages or to amino acids and peptides via amide linkages.

Free IAA is readily available as a commercial product. It may be synthesised chemically or prepared in a biological way. IAA producing micro-organisms are widespread in nature. Yeast, fungi and many bacteria as well as plants are known to convert precursors of IAA into free IAA. In addition to the L-tryptophan conversion by bacteria, also L-tryptophan independent biochemical routes towards free IAA are described extensively (J. Plant Growth Regul (2001) 20: 198-216).

A well known bacterium, capable of producing free IAA is Azospirillum Brasilens (AB). At the end of the growth phase in a regular fermentation process, AB is able to convert L-tryptophan into free IAA. To increase the efficiency of this conversion, a small amount of synthetic free IAA may be added to the media. Via a feedback mechanism, AB increases the conversion of L-tryptophan into free IAA.

Final concentrations of 1 gram free IAA / liter culture broth are easy to make, but even much higher concentrations are possible, depending on the micro-organism used.

After ending the fermentation the micro-organism may be lysed and a powder enriched in free IAA may be obtained by spray drying or any other convenient way of drying the culture broth. Other techniques may be used to remove liquids partly or completely.

As long ago as 1956, the effects of free IAA on humans were studied, and it was shown that single doses of 0.1 g/kg body weight were non-toxic (Mirsy A and Diengott D, Hypoglycemic action of indole-3-acetic acid by mouth in patients with diabetes mellitus, Proc. Soc. Exp. Biol. Med. 93: 109-110.1956). In 1964, it was found that photo-oxidation products of free IAA acted as growth inhibitors of micro-organisms (Still C, Fukuyama T and Moyed H, Inhibitory Oxidation Products of Indole-3-acetic acid, J. Biological Chemistry, 240.6,2612-2618,1964).

Also, the medical use of free IAA and some of its derivatives has previously been described. EP 1.296.676 describes the use of free IAA as a pharmaceutical, in particular for treating neoplastic disease in humans. WO 02/080906
describes the use of free IAA for treating endometriosis in women. Nachson et al. (Feed and Chemical Toxicology 41, 745-752) reported the effect of some free IAA derivates (indole-3-carbinol and 3,3'-diindolylmethane) on the proliferation and induction of apoptosis in human prostate cancer cell lines whereas Rossiter et al. (Bioorganic & Medicinal Chemistry Letters, 12, 2523-2526) as well as Folkes et al. (Biochemical Pharmacology 63, 265-272) described the use of free IAA and some derivatives in enzyme-prodrug directed cancer therapies.

Phytohormones and beta-glucans appeared to work in a wide range of concentrations for improving the speed of wound healing in animals. The optimal concentrations may vary between different species, however, the skilled person will know how to obtain an optimal concentration for a given species, for instance by titration of the desired compound into the animal feed and testing when this would have the optimal effect. The following may serve as guidance in this process.

A skilled person will appreciate that the amount of free IAA in the ready to use feed has to be adjusted in order to supply the animal with an effective amount of free IAA. In order to adjust the free IAA concentration in the feed so that a certain daily intake of free IAA is achieved, an estimate has to be made of the feed intake of an animal or animal group. A skilled person is aware of the feed intake of a (particular kind or group of) animal(s), typically, the feed intake per day is between 0.5 and 10% of the body weight of the animal, with occasional exceptions as high as 20%. Elderly animals tend to eat less and are considered to have a feed intake per day between 0.1 and 5%, typically of 1% of their body mass.

It was found that wounded animals recovered better and quicker from their injuries when free IAA was provided in their feed so that their daily intake was in the range of 0.004 and 40 mg per kilogram life weight per day (mg/kglw/day). Optimum between cost and benefit was reached in concentrations between 0.04 and 4 mg/kglw/day, in particular feed with 0.4 mg/kglw/day free IAA was very effective.

Therefore, in one aspect the invention relates to a method of treating the injured animal body in order to improve wound healing by administering the animal between 0.004 and 40 mg free IAA per kilogram life weight per day, preferably between 0.04 and 4 mg/kglw/day, more preferably 0.4 mg/kglw/day.

One particular good way of administering the free IAA to the animal is in an animal feed comprising between 1 and 100 milligrams of free IAA per kg feed, preferably between 10 and 100 milligrams per kg feed.

In another aspect the invention therefore relates to the use of free IAA
for the preparation of a medicament for increasing the speed of wound healing in injured animals. Preferably, such a medicament comprises free IAA in concentrations suitable for a treatment directed to a daily intake of between 0.004 and 40 mg free IAA per kilogram life weight per day, preferably between 0.04 and 4 mg/kglw/day, more preferably 0.4 mg/kglw/day, such as between 1 and 100 milligrams of free IAA per kg.

Animal feed comprising IAA has been described in the art, for instance, US-A-2925341 discloses a feed additive which comprises 10-50 mg of indole acetic acid per kilogram of feed.

The effect of improving the speed of recovery and wound healing was also observed when the feed of injured animals was supplemented with gibberellic acid. The optimal concentrations here were found to be within the range of 0.0004 and 4 mg/kglw/day. The effect of improving the recovery speed of wounded animals was particularly pronounced in the range of 0.004 and 0.4 mg/kglw/day of gibberellin. Optimal results were achieved between 0.01 and 0.1 mg/kglw/day, such as 0.04 mg/kglw/day.

Therefore, in one aspect the invention relates to a method of treating the injured animal body in order to improve wound healing and/or improving the speed of recovery by administering the animal between 0.0004 and 4 mg/kglw/day of gibberellin, preferably between 0.004 and 0.4 mg/kglw/day, even more preferably between 0.01 and 0.1 mg/kglw/day, such as 0.04 mg/kglw/day.

One particular good way of administering gibberellin to the animal is in an animal feed comprising between 0.1 and 100 milligrams of gibberellin per kg feed, preferably between 1 and 10 milligrams per kg feed.

In another aspect the invention therefore relates to the use of gibberellin for the preparation of a medicament for increasing the speed of wound healing and/or increasing the speed of recovery in injured animals. Preferably, such a medicament comprises gibberellin in concentrations suitable for a treatment directed to a daily intake of between 0.0004 and 4 mg/kglw/day of gibberellin, preferably between 0.004 and 0.4 mg/kglw/day, even more preferably between 0.01 and 0.1 mg/kglw/day, most preferably 0.04 mg/kglw/day, such as between 1 and 10 mg/kg feed.

Animal feed compositions comprising gibberellin are readily available in the art. US-A-2943938 and Svihus et al. (Journal of Animal Science, 64, 1997, p257-272) describe an animal feed which may comprise suitable amounts of gibberellic acid per kilogram of composition.

US 6174541 described the effects of IAA and gibberellin and their
derivatives on the migration of fibroblasts *in vitro*. It was found that IAA but not gibberellin had an effect on the migration of fibroblasts *in vitro*. The effects observed in our present study must therefore be caused by another mechanism than fibroblast migration.

It was also found that the effects of the above mentioned phytohormones could be enhanced by the addition of beta-glucans. In particular 1,3 and 1,6 beta glucans were very useful to improve the speed of recovery and/or the speed of wound healing of wounded animals.

When wounded animals were fed with 1 to 1000 mg/kg/lw/day of dried *Agaricus blazei* murill, this was found to produce the desired effect of improving the speed of recovery of wounded animals. This corresponds to approximately 0.1 to 100 mg/kg/lw/day of 1,3 and 1,6 beta glucans. Excellent results were obtained with feeding the animals between 1 to 10 mg/kg/lw/day of 1,3 and 1,6 beta glucans, optimum of cost benefit was found to be around 5 mg/kg/lw/day, corresponding to 50 mg/kg/lw/day of dried ABM. Preferably the 1,3 and 1,6 beta glucans are purified 1,3 and 1,6 beta glucans.

Therefore, in one aspect the invention relates to a method of treating the injured animal body in order to improve wound healing and/or improving the speed of recovery by administering the animal between 0.1 to 100 mg/kg/lw/day of 1,3 and 1,6 beta glucans, preferably between 1 to 10 mg/kg/lw/day, more preferably 5 mg/kg/lw/day.

One particular good way of administering 1,3 and 1,6 beta glucans to the animal is in an animal feed comprising between 0.05 and 500 milligrams 1,3 and 1,6 beta glucans of per kg feed, preferably between 0.5 and 50 milligrams per kg feed, such as between 1 and 10 milligrams per kg feed.

In another aspect, the invention therefore relates to the use of 1,3 and 1,6 beta glucans for the preparation of a medicament for increasing the speed of wound healing and/or increasing the speed of recovery in injured animals. Preferably, such a medicament comprises 1,3 and 1,6 beta glucans in concentrations suitable for a treatment directed to a daily intake of between 0.1 to 100 mg/kg/lw/day of 1,3 and 1,6 beta glucans, preferably between 1 to 10 mg/kg/lw/day, more preferably of 5 mg/kg/lw/day.

A particularly good source of such 1,3 and 1,6 beta glucans may be found in preparations of *Agaricus blazei* murill or yeast cell walls.

animal feed compositions comprising beta-glucans that may be suitable for use in the present invention.

Surprisingly, it was observed that the combination of phytohormones with beta-glucans had a synergistic effect. The effects of the combination treatment appeared to be better than could be expected from the results of the individual treatments.

As a consequence, the invention is therefore also directed towards the use of any of the substances described above, for the preparation of a medicament for the treatment of wounded animals in order to improve the speed of recovery and/or in order to accelerate wound healing.

REFERENCES


**EXAMPLES**

**Example 1 Microbiological production of a preparation containing free IAA**

Azospirillum brasilense Sp7 (ATCC) was obtained as an agar culture in a culture tube. LB medium was used to grow the strain overnight at 28 °C at 175 rpm. Glycerol was added to the culture up to 10 %, mixed and divided over Nalgene creovials and frozen at – 80 °C. Stocks were stored at – 80 °C in creovials.

To prepare a seed culture of A. brasilense, one stock (1.2 to 1.8 ml) was thawed and added to 1 liter of LB medium and grown for about 20 h at 28 °C and 175 rpm to an Optical Density (OD620 nm) of about 2.5.

A 10 litre fermentor was rinsed with water and the pH electrode was calibrated. Nine litre of LB medium was prepared and 1 g/l L-Tryptophan and 0.1 g/l free IAA was added. The medium was entered into the fermentor together with 2 ml of anti foam. The fermentor was sterilised for 30 min at 121 °C. After cooling down to 28 °C, the O2 probe is calibrated with N2 and O2, 0 and 100 % air saturation respectively.

The seed culture is transferred to the fermentor via a flask and tubing which are separately sterilised in an autoclave. When the addition is completed the tubing and flask are removed and the fermentation is started with the following parameters:

- Stirrer speed: 400 rpm
- Temperature: 28 °C
- Aeration: 0.75 NL/min
- PH 7

After 15 min a sample is taken to measure the OD620 nm and check the pH. Samples are taken at certain intervals to quantify the growth of A. brasilense. When the growth rate declined extra medium was added to ensure that enough biomass was formed for the production of free IAA. It was found that the production of free IAA started when the active growth phase ended and continued for a prolonged period. The course of the free IAA concentration was followed by LC-MS. When the concentration of
free IAA was at a level of about 1 g/l, the fermentation was terminated and the cells were harvested and lysed by means of a nonojet homogeniser at about 1400 bar. The remaining supernatant and the lysed cells were sterilised and spray dried to yield the desired product formulation.

Example 2 Preparation of dog feed containing beta glucans

An amount of 5.0 gram of dried Agaricus Blazei Murill (Agaricus Farm), a natural source of beta-glucans was suspended in 100 ml of olive oil. A dog feed according to the invention was prepared by vacuum impregnating one kilogram of commercially available Royal Canin Mini Adult feed with 100 ml of the oil suspension. Control feed was prepared by vacuum impregnating the same amount of feed with only olive oil.

Example 3 Preparation of dog feed containing plant growth hormones

An amount of the spray dried formulation as described in example 1 corresponding to 40 milligram of free IAA was suspended in 100 ml of olive oil. A dog feed according to the invention was prepared by vacuum impregnating one kilogram of commercially available Royal Canin Mini Adult feed with 100 ml of the oil suspension. Control feed was prepared by vacuum impregnating the same amount of feed with only olive oil.

Example 4 Preparation of dog feed containing both beta glucans and plant growth hormones

An amount of 5.0 gram of dried Agaricus Blazei Murill (Agaricus Farm), a natural source of beta-glucans and an amount of the spray dried formulation as described in example 1 corresponding to 40 milligram of free IAA were suspended in 100 ml of olive oil. A dog feed according to the invention was prepared by vacuum impregnating one kilogram of commercially available Royal Canin Mini Adult feed with 100 ml of the oil suspension. Control feed was prepared by vacuum impregnating the same amount of feed with only olive oil.

Example 5 Improving the recovery speed of dogs.

An experiment was set up to test the efficacy of the food according to the invention. In a large animal clinic, all owners of dogs that came in for a pyometra operation were asked to participate in a double-blind study. The word "pyometra" is
derived from latin "pyo" meaning pus and "metra" meaning uterus. The pyometra is an abscessed, pus-filled infected uterus. Toxins and bacteria leak across the uterine walls and into the bloodstream causing life-threatening toxic effects, Without treatment death is inevitable.

Classically, the patient is an older female dog. Usually, she finished a heat cycle in the previous 1-2 months. She had a poor appetite and may be vomiting or drinking an excessive amount of water. In the more usual "open pyometra" the cervix is open and the purulent uterine contents is able to drip out thus a smelly vaginal discharge is usually apparent.

Participants to the study were given a recovery food according to either example 2, 3 or 4. A control group received the control feed. After one year, 124 dogs had participated in the study. The veterinarians filled in a questionnaire on the recovery process of the dogs. Criteria were the speed of disappearance of fever, reoccurrence of appetite, mobility and activity of the animal and actual healing of the surgical incision. These criteria were scored on a scale ranging from 1 to 5 wherein 1 was very slow and 5 very fast. These criteria were scored 1, 2, 4 and 7 days post-surgery and 3 weeks post surgery.

After one year of study, the code was broken and the results were analysed. It appeared that animals that had received feed according to examples 2 and 3 both performed significantly better than the animals that had received the control feed. After 3 weeks, no differences were found anymore between the groups. The group that received feed according to example 4 outperformed even the results of all the other groups in that their recovery was even faster. Again, after 3 weeks no significant difference was found anymore.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Post surgery feed additives for dog food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.4 mg/kg/lw/day free IAA</td>
</tr>
<tr>
<td>Group 2</td>
<td>50 mg/kg/lw/day of dried ABM corresponding to 5 mg/kg/lw/day of 1,3 – 1,6 beta glucan</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.4 mg/kg/lw/day free IAA plus 50 mg/kg/lw/day of dried ABM corresponding to 5 mg/kg/lw/day of 1,3 – 1,6 beta glucan</td>
</tr>
<tr>
<td>Group 4</td>
<td>none</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
</tr>
</tbody>
</table>
Example 6: Preparation of fish feed containing beta glucans

An amount of 3.0 gram of Agaricus Blazei Murill (Agaricus Farm), a natural source of beta-glucans was suspended in 100 ml of olive oil. A fish feed according to the invention was prepared by vacuum impregnating one kilogram of commercially available (Coppens) Cyprico White 3 mm floater feed with 100 ml of the oil suspension. Control feed was prepared by vacuum impregnating the same amount of feed with only olive oil.

Example 7: Preparation of fish feed containing plant growth hormones

An amount of the spray dried formulation as described in example 1 corresponding to 12 milligram of free IAA was suspended in 100 ml of olive oil. A fish feed according to the invention was prepared by vacuum impregnating one kilogram of commercially available (Coppens) Cyprico White 3 mm floater feed with 100 ml of the oil suspension. Control feed was prepared by vacuum impregnating the same amount of feed with only olive oil.

Example 8: Preparation of fish feed containing both beta glucans and plant growth hormones

An amount of 3.0 gram of Agaricus Blazei Murill (ABM, Agaricus Farm), a natural source of beta-glucans and an amount of the spray dried formulation as described in example 1 corresponding to 12 milligram of free IAA were suspended in 100 ml of olive oil. A fish feed according to the invention was prepared by vacuum impregnating one kilogram of commercially available (Coppens) Cyprico White 3 mm floater feed with 100 ml of the oil suspension. Control feed was prepared by vacuum impregnating the same amount of feed with only olive oil.

Example 9: Use of fish feed comprising beta glucans and plant growth hormones to improve the speed of recovery of wounds

Four ponds of 40 cubic meters each, each containing 50 koi fish with an approximate total body weight of 50 kg were used to establish the effect of the fish feed according to the invention on wound healing. One pond (pond 1) served as a control were the fish were fed with control feed, the fish in the other ponds received a feed according to the invention as prepared in examples 6 to 8 above.

The fish in control pond were fed with 10 gram Cyprico White 3 mm floater feed per day, whereas the fish in the other ponds were fed with 10 gram of the feed as
described in Example 2, 3 and 4 (Table 2). About 10% of all fish suffered from wounds as a result of parasites.

The veterinarian filled in a questionnaire on the recovery process of the fish, Table 3. Criterion was the speed of disappearance of the wounds. This criterion was scored on a scale ranging from 1 to 5 wherein 1 was very slow recovery and 5 very fast recovery. This criterion was scored 1, 2, 4, 7 and 21 days of feeding.

**Table 2**

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<td>Pond 3</td>
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<td>Pond 4 (control)</td>
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**Table 3**

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CLAIMS

1. A method for improving the speed of recovery and/or the speed of wound healing of an animal body by administering the animal with an effective amount of a phytohormone.

2. A method according to claim 1 wherein the phytohormone is free IAA.

3. A method according to claim 2 wherein the free IAA is administered in a dose between 0.004 and 40 mg of free IAA per kilogram live weight per day, preferably between 0.04 and 4 mg/kglw/day, more preferably 0.4 mg/kglw/day.

4. A method according to claim 1 wherein the phytohormone is gibberellin or gibberellic acid.

5. A method according to claim 4 wherein the gibberellin or gibberellic acid is administered in a dose between 0.0004 and 4 mg/kglw/day, preferably between 0.004 and 0.4 mg/kglw/day, even more preferably between 0.01 and 0.1 mg/kglw/day, such as 0.04 mg/kglw/day.

6. A method according to claims 1 – 5 wherein the phytohormone is supplemented with 1,3 – 1,6 beta-glucans.

7. A method according to claim 6 wherein the beta-glucans are administered in a dose between 0.1 to 100 mg/kglw/day, preferably between 1 to 10 mg/kglw/day, more preferably in a dose of 5 mg/kglw/day.

8. Use of an animal feed according to claims 1 to 7 for the preparation of a medicament for improving the speed of recovery and/or the speed of wound healing in animals.

9. Animal feed comprising between 1 and 100 mg free IAA per kg feed and between 0.05 and 500 mg beta-glucans per kg feed.

10. Animal feed comprising between 0.1 and 100 mg gibberellin per kg feed and between 0.05 and 500 mg beta-glucans per kg feed.
A. CLASSIFICATION OF SUBJECT MATTER

INV. A61P17/02 A23K1/18 A23K1/16

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)
A23K

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)
EPO-Internal, WPI Data, FSTA, CAB Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<td>X</td>
<td>US 6 174 541 B1 (SONG JIN ET AL) 16 January 2001 (2001-01-16) column 2, lines 58,59; claim 1; figures 1-5</td>
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<td>X</td>
<td>Further documents are listed in the continuation of Box C.</td>
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X See patent family annex.

Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document 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.
- "&" document member of the same patent family

Date of the actual completion of the International search: 17 August 2006

Date of mailing of the international search report: 08/09/2006

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fac. (+31-70) 340-3016

Authorized officer: Rooney, K
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<td>WO 02/37988 A (JAN KOYNAR-PERFEKTRA; KOYNAR, JAN; NOVAK, MIROSLAV; GRULICH, VACLAV; P) 16 May 2002 (2002-05-16) page 2, paragraph 3; claim 1</td>
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<td>WO 02/091850 A (SUOMEN REHU OY; VUORENMAA, JUHANI; RAUTONEN, NINA) 21 November 2002 (2002-11-21) page 6, paragraph 2; claims 1, 8</td>
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<td>WO 2004/066863 A (IMMUDYNE, INC; SORGENTE, NINO; COURIE, JR., PHILLIP, A; MILES, AMY, J) 12 August 2004 (2004-08-12) claims 13, 24; example 3</td>
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<td>WO 2004/014320 A (BIOPOLYMER ENGINEERING, INC; OSTROFF, GARY, R; ROSS, GORDON, D; UNIVER) 19 February 2004 (2004-02-19) page 23, paragraph 5 - page 24, paragraph 2</td>
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<td>US 5 702 719 A (DONZIS ET AL) 30 December 1997 (1997-12-30) column 2, line 65 - column 3, line 30 examples 3,4</td>
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<td>WO 01/64205 A (THE INSTITUTES FOR PHARMACEUTICAL DISCOVERY, LLC; SREDY, JANET; VAN ZA) 7 September 2001 (2001-09-07) examples 3,37</td>
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INTERNATIONAL SEARCH REPORT

Box II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.: — because they relate to subject matter not required to be searched by this Authority, namely:

   Although claims 1-7 is directed to a method of treatment of the animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the composition.

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.

Form PCT/SA/210 (continuation of first sheet (2)) (January 2004)
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