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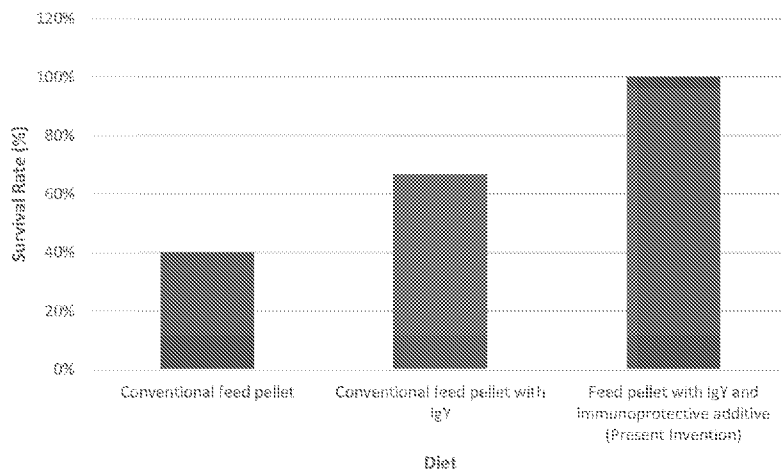


FIGURE 1

(57) Abstract: The present invention relates to a composition for an aquaculture feed. More particularly, the present invention relates to an aquaculture feed having immunotherapeutic properties. The composition comprising a carbohydrate, an immunoglobulin Y, wherein the immunoglobulin Y is in an amount of 3 to 10% weight/weight, wherein the immunoglobulin Y is derived from an egg yolk of a pathogen-immunized egg-laying chicken, and an immune-enhancing additive for protecting the immunoglobulin Y from degradation action of a host's digestive enzymes and pH conditions. The composition possesses immune-enhancing property and capable of boosting innate immune system of the aquaculture hosts or animals.



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A COMPOSITION FOR AN IMMUNE-ENHANCING AQUACULTURE PELLETField of the Invention

5 The present invention relates to a composition for use in an aquaculture feed.

Background of the Invention

10 In 2015, global aquaculture production reached 106 million tonnes while feeding materials demanded by the aquaculture industry has reached 39.9 million tonnes. On the other hand, infectious microbial diseases continue to impose major yield-limiting factors on the aquaculture production with the overall global impact of these diseases exceeding \$6 billion per annum. It is a challenge for the industry to
15 find a way of increasing aquaculture production while overcoming the infectious microbial diseases with limited costs and resources.

 Conventionally, a method for increasing the aquaculture production is by increasing the weight of the aquaculture products. This involves increasing muscle
20 mass and/or fat mass of the aquaculture animals and reducing risk of diseases that could disturb the growth of the aquaculture animals. Fish and crustaceans are typically fed with a high protein and carbohydrate diet and injected or immunised with an antibody that would help to increase their body weight and at the same time reducing infectious diseases. However, this method is too impractical and stressful
25 to the aquaculture animals or hosts. Several methods have been developed to incorporate antibody with animal feeds.

 CA2667412C is a patent publication that discloses feed materials for improving the growth of a recipient, particularly in aquaculture animals. The
30 invention describes counteracting of the negative nutritional effect of soy bean meal by adding manna-oligosaccharides (MOS) to the diet. Further improvements in growth were observed when MOS was fed in combination with added nucleotides and an immune stimulant. The invention also discloses improved pigment uptake in fish fed compounds of the present invention.

US20080175851A1 discloses a composition comprising fish or shellfish food and an antibody that reduces the bioavailability of a prostaglandin or leukotriene lipid precursor, wherein the antibody is present in an effective amount for increasing weight gain or feed efficiency in fish and shellfish administered the antibody
5 compared to fish or shellfish not administered the antibody.

It was reported that some feeding compositions that are incorporated with the antibody tends to degrade as the antibody is vulnerable to degradation action of the host's digestive enzymes and pH conditions. Hence, there appears a need for
10 the present invention to solve the above problem.

Summary of the Invention

The present invention relates to a composition for use in an aquaculture feed.
15 More particularly, the present invention relates to an aquaculture feed having immunotherapeutic properties.

Accordingly, in an aspect of the present invention, there is provided a composition of an aquaculture feed, characterized in that, the composition
20 comprising a carbohydrate, an immunoglobulin Y, wherein the immunoglobulin Y is in an amount of 3 to 10% weight/weight, wherein the immunoglobulin Y is derived from an egg yolk of a pathogen-immunized egg-laying chicken, and an immune-enhancing additive for protecting the immunoglobulin Y from degradation action of a host's digestive enzymes and pH conditions.

25

The carbohydrate and the immune-enhancing additive are derived from starch. The starch is in an amount of 18 to 40% weight/weight of the composition. The immune-enhancing additive is an immune-enhancing additive produced by a process comprising steps of mixing a pre-processed immune-enhancing additive
30 with 10 to 80% water, heating the mixture under a temperature of between 60 to 120°C for 1 to 30 minutes under constant stirring, drying the mixture under a temperature of between 10 to 100°C to form a solid, and grinding the dried solid into smaller particles with a hammer mill.

The composition is suitable for use in a basal diet. The basal diet is selected from a basal diet comprising minerals, vitamins, vegetable oil, fish meal and soybean meal. The minerals and the vitamins are in an amount of 1 to 20% weight/weight of the composition. The vegetable oil is in an amount of 1 to 20% weight/weight of the composition. The fish meal is in an amount of 5 to 30% weight/weight of the composition. The soybean meal is in an amount of 5 to 30% weight/weight of the composition.

It is an object of the present invention to provide a composition of an aquaculture feed that utilizes additive that possess immune-enhancing property and capable of boosting innate immune system of the aquaculture hosts or animals.

The present invention consists of certain novel features and a combination of parts hereinafter fully described and illustrated in the accompanying drawings and particularly pointed out in the appended claims; it is being understood that various changes in the details may be without departing from the scope of the invention or sacrificing any of the advantages of the present invention.

Brief Description of the Drawings

For the purpose of facilitating an understanding of the invention, there is illustrated in the accompanying drawings the preferred embodiments from an inspection of which when considered in connection with the following description, the invention, its construction and operation and many of its advantages would be readily understood and appreciated.

Figure 1 is a graph illustrating survival rates of tilapia fed with different diets after 72 hours of bacteria challenge test according to the present invention.

Figure 2 is a graph illustrating survival rates of *Macrobrachium rosenbergii* fed with different diets after 24 hours of bacteria challenge test according to the present invention.

Detailed Description of the Invention

The present invention relates to a composition for use in an aquaculture feed. More particularly, the present invention relates to an aquaculture feed having immunotherapeutic properties. Hereinafter, the composition of the feed shall be described according to the preferred embodiments of the present invention and by referring to the accompanying description and drawing(s). However, it is to be understood that limiting the description to the preferred embodiments of the invention and to the drawings is merely to facilitate discussion of the present invention and it is envisioned that those skilled in the art may devise various modifications without departing from the scope of the appended claim.

The term "immunoglobulin Y" or "IgY" refers to a type of immunoglobulin which is the major antibody in bird, reptile, and lungfish blood. It is also found in high concentrations in chicken egg yolk. As with the other immunoglobulins, IgY is a class of proteins which are formed by the immune system in reaction to certain foreign substances, and specifically recognize them.

The present invention will now be described in further detail by way of preferred embodiments and non-limiting example(s).

Accordingly, in an aspect of the present invention, there is provided a composition of an aquaculture feed, characterized in that, the composition comprising a carbohydrate, an immunoglobulin Y, wherein the immunoglobulin Y is in an amount of 3 to 10% weight/weight, wherein the immunoglobulin Y is derived from an egg yolk of a pathogen-immunized egg-laying chicken, and an immune-enhancing additive for protecting the immunoglobulin Y from degradation action of a host's digestive enzymes and pH conditions.

Preferably, the carbohydrate and the immune-enhancing additive are derived from starch. The starch is preferably present in an amount of 18 to 40% weight/weight of the composition.

It is also preferred that the immune-enhancing additive is an immune-enhancing additive produced by a process comprising steps of mixing a pre-

processed immune-enhancing additive with 10 to 80% water, heating the mixture under a temperature of between 60 to 120°C for 1 to 30 minutes under constant stirring, drying the mixture under a temperature of between 10 to 100°C to form a solid, and grinding the dried solid into smaller particles with a hammer mill.

5

In another embodiment, it is preferred that the composition is suitable for use in a basal diet. Preferably, the basal diet is selected from a basal diet comprising minerals, vitamins, vegetable oil, fish meal and soybean meal. Preferably, the minerals and the vitamins are in an amount of 1 to 20% weight/weight of the composition. It is also preferred that the vegetable oil is in an amount of 1 to 20% weight/weight of the composition, the fish meal is in an amount of 5 to 30% weight/weight of the composition and the soybean meal is in an amount of 5 to 30% weight/weight of the composition.

It is an advantage of the present invention to provide a composition of an aquaculture feed that utilizes additive that possess immune-enhancing property and capable of boosting innate immune system of the aquaculture hosts or animals.

The present invention will now be described in further detail by way of an example.

EXAMPLE 1

In this example, an aquaculture feed pellet with an immune-enhancing additive is described.

The current pellets contain 5-8% (w/w) egg yolk from the target pathogen-immunised hen. The starch was prepared with the novel process to be added into pellet to serve as source of carbohydrate and as an additive to protect the egg yolk IgY from the degrading activities of the fish digestive enzymes and pH. The pellet can be used to feed the aquaculture candidates ad libitum or 1-4% body mass 1-3 times daily.

The additive has been proven to enhance energy bioavailability of the fishes. The feed intake of the fishes fed with the current diets showed significantly

lower food intake but yielded similar weight gains and growth as compared to the conventional diets. Better digestion reflected by a higher liver glycogen and lipid content of the animal was observed. The diet with the current additive resulted in significantly higher phagocytosis activities and respiratory assay in the in vitro assays.

With reference to Figure 1, an immersion challenge was conducted with 5×10^7 CFU per L of *Aeromonas hydrophila* (the dosage was determined by LC_{50}). After 72 hours, 100% survival rate was observed in the tilapia (*Oreochromis niloticus*) fed with the aquaculture feed pellet with an immune-enhancing additive for 1-week duration as compared to 40% in that of the conventional diet group. Without the additive, the presence of IgY alone was only resulted in 66.7% of survival rate. Similarly, with reference to Figure 2, 100% of survival rate was observed in the giant freshwater prawns (*Macrobrachium rosenbergii*) that fed with the aquaculture feed pellet with an immune-enhancing additive upon immersion challenge with 9×10^7 CFU per L of *Aeromonas hydrophila*, as compared to 100% mortality in the prawns that fed with the conventional diet.

The underlying mechanisms of the immunoprotection of IgY include the clumping the bacteria together with the antibody-antigen interaction, in turn facilitate the clearance of bacteria by phagocyte. The IgY also capable to neutralise the toxin produce by the target pathogen as well as blockage of active sites of the target pathogen to prevent them to attach and invade the host's mucosa.

Hen yolk IgY antibodies are very vulnerable to enzymatic degradation of pepsin and low pH of digestive juices of the aquaculture candidates. The new, effective and economic way to preserve the immunoprotective effect of IgY as described herein is very useful in the production of feed pellet. In addition, the additive that are used to preserve the IgY possess immune-enhancing property that is capable of boosting innate immune system of aquaculture species. Addition of these ingredients in the basal diet, provide additional immunoprophylactic effect to the aquaculture species. This feed proves to be an excellent feed for aquacultures across different fish and crustacean species.

The feed pellet can be stored in room temperature for a storage period of up to 12 months. For longer storage, it should be kept in a temperature of 4 °C.

Industrial Application

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The present invention is industrially applicable. The composition of the present invention is capable in improving pathogenic resistance and enhance innate immune responses as well as increase feed conversion rate in an aquaculture host. Hence, it is very useful in the fishing industry.

10

While in the foregoing specification this invention has been described in relation to certain preferred exemplary embodiments thereof and many details have been set forth for purpose of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein can be varied considerably without departing from the basic principles of the invention.

15

CLAIMS

1. A composition of an aquaculture feed, characterized in that, the composition comprising
5 a carbohydrate;
an immunoglobulin Y, wherein the immunoglobulin Y is in an amount of 3 to 10% weight/weight, wherein the immunoglobulin Y is derived from an egg yolk of a pathogen-immunized egg-laying chicken; and
an immune-enhancing additive for protecting the immunoglobulin Y from
10 degradation action of a host's digestive enzymes and pH conditions.
2. The composition according to claim 1, wherein the carbohydrate is derived from starch.
- 15 3. The composition according to claim 1, wherein the immune-enhancing additive is derived from starch.
4. The composition according to claim 3, wherein the starch is in an amount of 18 to 40% weight/weight.
20
5. The composition according to claim 1, wherein the immune-enhancing additive is an immune-enhancing additive produced by a process comprising steps of:
 - i) mixing a pre-processed immune-enhancing additive with 10 to 80% water;
 - 25 ii) heating the mixture of step i under a temperature of between 60 to 120°C for 1 to 30 minutes under constant stirring;
 - iii) drying the mixture under a temperature of between 10 to 100°C to form a solid; and
 - iv) grinding the dried solid into smaller particles with a hammer mill.
30
6. The composition according to claim 1, wherein the composition is suitable for use in a basal diet.

7. The composition according to claim 6, wherein the basal diet is selected from a basal diet comprising minerals, vitamins, vegetable oil, fish meal and soybean meal.

5 8. The composition according to claim 7, wherein the minerals and the vitamins are in an amount of 1 to 20% weight/weight.

9. The composition according to claim 7, wherein the vegetable oil is in an amount of 1 to 20% weight/weight.

10

10. The composition according to claim 7, wherein the fish meal is in an amount of 5 to 30% weight/weight.

11. The composition according to claim 7, wherein the soybean meal is in an
15 amount of 5 to 30% weight/weight.

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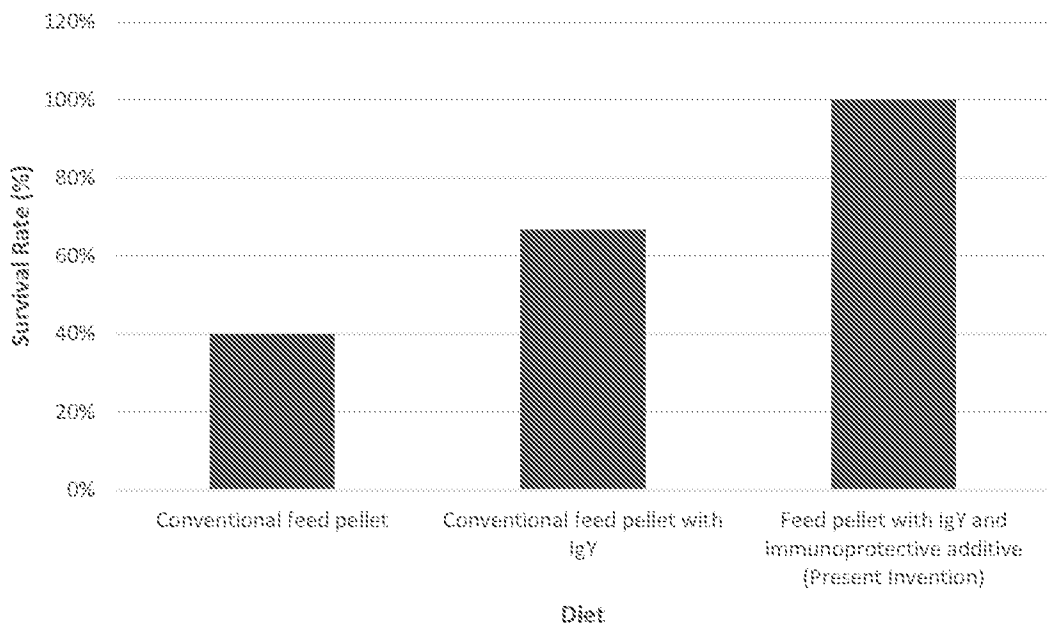


FIGURE 1

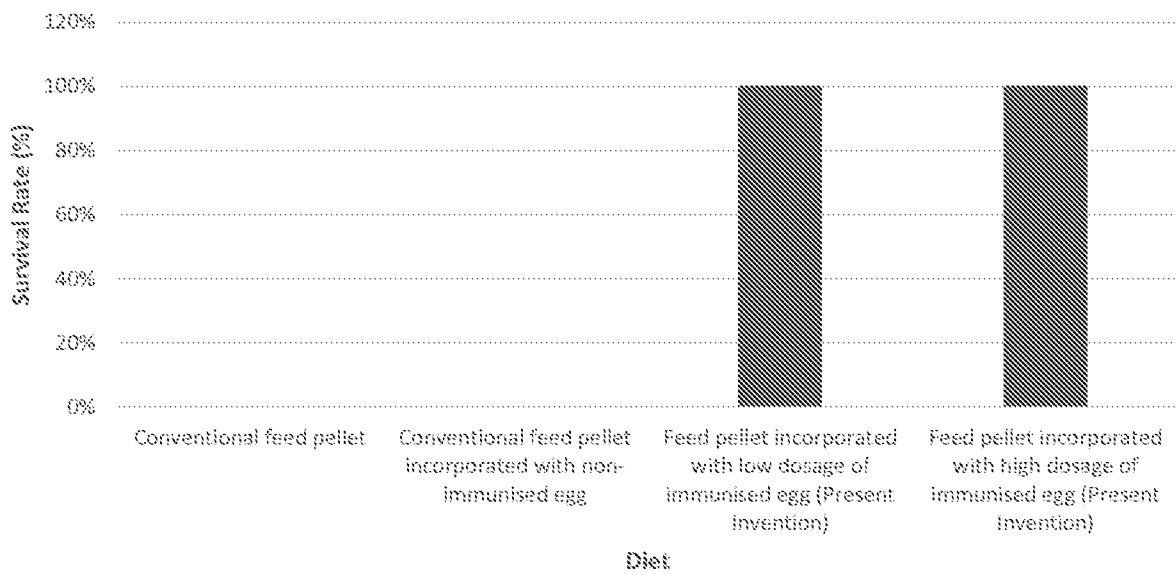


FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2019/050131**A. CLASSIFICATION OF SUBJECT MATTER****A23K 20/147(2016.01)i, A23K 20/105(2016.01)i, A23K 20/158(2016.01)i, A23K 20/174(2016.01)i, A23K 40/10(2016.01)i, A23K 50/80(2016.01)i, A23K 10/22(2016.01)i, A23K 10/30(2016.01)i**

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 20/147; A23K 10/22; A23K 50/80; C02F 3/34; A23K 20/105; A23K 20/158; A23K 20/174; A23K 40/10; A23K 10/30

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: aquaculture feed, carbohydrate, immunoglobulin Y, additive, egg yolk

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOVACS-NOLAN J. et al., 'Egg yolk antibodies for passive immunity' , Annu. Rev. Food Sci. Technol, 2012 [E-pub.: 28 November 2011], Vol. 3, pp. 163-182 abstract; page 168, lines 1-11	1-11
A	BALOGH, A. R. et al., 'IgY Technology in aquaculture-a review' , Reviews in Aquaculture, 2015, Vol. 7, No. 3, pp. 153-160 abstract; pages 154-156	1-11
A	CHALGHOUMI, R. et al., 'Hen egg yolk antibodies (IgY), production and use for passive immunization against bacterial enteric infections in chicken: a review' , Biotechnol. Agron. Soc. Environ, 2009, Vol. 13, No. 2, pp. 295-308 abstract; pages 298-301	1-11
A	CN 108850688 A (THE RICH MARINE ORGANISMS PRODUCTS CO., LTD.) 23 November 2018 the entire document	1-11
A	CN 102963985 A (JIANGSU HONGJI BIOLOGICAL SCIENCE & TECHNOLOGY CO., LTD.) 13 March 2013 the entire document	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

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"&" document member of the same patent family

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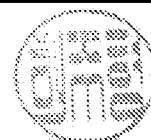
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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CN 108850688 A

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None

CN 102963985 A

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