

METHODS OF MANGROVE LEAF USE, FORMULATION AND PREPARATION

Natural mangrove leaf use, formulation, and preparation as an additive in feeds and feed supplements for commercial shrimp and fish aquaculture. The use of mangrove leaves of the *Avicennia* and *Rhizophoraceae* families as an aquaculture feed ingredient formulated into a compound feed for use to improve aquaculture pond water quality, animal health, disease immunity, and growth.

[0001] The present invention relates to a feed additive composition for improving shrimp health and growth comprising mangrove leaves of the *Rhizophoraceae* family as an active ingredient and a feed comprising the same.

[0002] The aquaculture industry is a major contributor to human protein requirements and has a much lower negative environmental footprint than terrestrial sources of protein. In 2020, the global aquaculture production was 178 million tons with most of this in less developed countries producing protein for domestic consumption.

Shrimp production comprises over 5 million tons of this production and is the largest traded global aquaculture commodity surpassing salmon with 3 million tons of annual production. This production is mainly comprised of two shrimp species of the *Penaeidae* family: Pacific white shrimp (*Penaeus vannamei*) and Giant tiger prawn (*Penaeus monodon*) with production dominated by *vannamei* here-in referred to as “shrimp”.

[0003] The shrimp aquaculture industry is beset with production challenges relating to disease. This is due to the rudimentary shrimp digestive and lack of cell memory and an adaptive immune system and the fact that modern aquaculture methods farm shrimp in high densities with many adjoining ponds making disease outbreaks spread rapidly throughout the farm and the region. Responsible farmers practice strict

biosecurity but diseases often spread thru water exchange and by other vectors such as birds, land crabs, even wind.

[0004] In many shrimp producing countries, farmers had often relied on the prophylactic use of banned antibiotics to keep their shrimp alive but increased testing
5 the rejection of shrimp containing antibiotic residues has encouraged farmers to find better alternatives. Now, many shrimp farmers use various probiotics and prebiotics to maintain shrimp health. Probiotics are defined as microbial feed additives that modulate gastrointestinal microbial communities whereas prebiotics are non-digestible forage additives stimulate the activity or abundance of beneficial
10 gastrointestinal bacteria.

[0005] In their natural habitat, shrimp are found in, and near mangrove estuaries and it has been thought that there is some beneficial effect that the mangrove forests are having on shrimp health, growth, and reproduction but which is little understood. In modern shrimp farms, this interaction is no longer there with the mangrove forests
15 often being destroyed for the establishment of the shrimp farms.

[0006] The inventor has observed integrated shrimp and mangrove aquaculture systems in Southern Vietnam and decided to incorporate them as an ingredient a shrimp feed supplement that he was developing.

[0007] Mangrove leaves of the species of *Avicennia marina* and *Rhizophora*
20 *apiculate* can be used and other mangrove species that are lacking of tannins or other anti-nutritional properties. Blending ratio can be from 0-100% of mangrove species selected. Mangrove leaves are selected and may be alive and green, dying and yellow, dried and brown, fixed or fallen.

[0008] Leaves are chopped to widths of 3 to 10 millimeters and lengths of the leaf
25 width. Leaves may be wet ground to a fine paste. Alternatively, leaves may be dried

and ground in a disk mill or in a hammer mill to a fine powder. Paste or particle size of the ground leaf is from a fine dust or paste to 0.5mm in diameter. If the leaves are dried when ground, water is added to yield a paste consistency.

[0009] Mangrove leaf paste is fermented with a mixture of bacteria and fungus in a ration from 0-100%, ideal ratio is 70% bacteria and 30% fungus. Paste is fermented in an air-inhibiting ridged container or flexible plastic bag. The fermentation time may vary from 50 hours up to 100 hours with the ideal time being 72 hours.

[0010] After fermentation, mangrove leaves are mixed with other ingredients which may be binders, fillers, and may contain seaweeds and starches to form a shrimp supplement product. Mixed dough is extruded into round or cylindrical pellets of equal width and length and may vary from a fine powder to 10 mm in diameter. Pellets may be formed using pressure, heat, or steam. Pellets are dried to a moisture content of 10% but may vary from 5% until 15% of total product weight.

[0011] Total inclusion of mangrove leaf in the supplement product is 3% by dry weight of total product composition but may vary from 1% to 5%. Total inclusion of shrimp supplement into the total feed composition to be fed to farmed shrimp is 5% but may vary from 2% up to 8% of total shrimp diet.

[0012] Feed trials were conducted with a shrimp supplement fed to shrimp at a 5% dietary replacement of conventional shrimp feed. The supplement contained 3% dry weight of fermented mangrove leaves. The total inclusion rate of ground, fermented, and dried mangrove leaves was 0.015% of the total shrimp feed diet. Shrimp were fed the supplement once per day along with the conventional feed diet being administered four times per day at regular intervals. Four diets were fed to identical shrimp ponds with Data on total weight gain, survival, and feed consumption

recorded every seven days. Shrimp were stocked at 12-day post larva and the trial ran for 67 days.

[0013] There was not a statistically significant between the control supplement formulation A without mangrove leaf inclusion and formulation D and E with 10% mangrove leaf inclusion or formulation F with 5% mangrove leaf inclusion demonstrating a lack of anti-nutritional elements in the mangrove leaf and shrimp feed supplement formulated from them.

[0014] In the following are described examples of preferred embodiments illustrated in the accompanying drawings, wherein:

- 10 Fig. 1 showing mangrove leaves;
- Fig. 2 showing harvested mangrove leaves;
- Fig. 3 showing chopped mangrove leaves;
- Fig. 4 shows ground mangrove leaves;
- Fig. 5 shows fermenting mangrove leaves;
- 15 Fig. 6 shows examples of beneficial effects of the invention;
- Fig. 7 shows further examples of beneficial effects of the invention; and
- Fig. 8 shows additional examples of beneficial effects of the invention.

Claims

1. Mangrove leaf inclusion in shrimp diet can have a positive effect of shrimp health, vigor, and disease resistance.
2. Mangrove leaf inclusion in shrimp diet may improve shrimp Survival Rates (SR), Average Daily weight Gain (ADG), and Feed Conversion Ratios (FCR).
3. Shrimp growth, feed additive composition for a shrimp supplement product comprising mangrove leaves of the *Rhizophoraceae* family as an active ingredient.
4. The feed additive composition of claim 3, wherein the feed additive has aquaculture pond water stability improvement, shrimp disease resistance, and shrimp growth improvement.
5. The feed additive composition of claim 3, comprising mangrove leaves in a proportion of 1 to 5% by dry weight of the total composition of the shrimp supplement.
6. The feed additive composition of claim 3, wherein the mangrove leaves are in powder form.
7. The feed additive composition of claim 3, wherein the mangrove leaves of the species of *Avicennia marina* and *Rhizophora apiculate* or other species of the *Rhizophoraceae* family are in a blending percentage from 0-100% of mangrove species selected.
8. An aquaculture feed supplement comprising the feed additive composition of any one of claims 1 to 7.
9. An aquaculture feed additive composition for improving aquaculture pond water stability, shrimp disease resistance, and shrimp growth improvement comprising mangrove leaves as an active ingredient.

10. A method of improving shrimp disease resistance and shrimp growth wherein mangrove leaves are an active ingredient.
11. The method of claim 10, wherein the mangrove leaves of the species of *Avicennia marina* and *Rhizophora apiculate* or other species of the
5 *Rhizophoraceae* family are in a blending percentage from 0-100% of mangrove species selected.
12. A method of improving the aquaculture pond water quality and stability wherein mangrove leaves are added to shrimp pond water.
13. The method of claim 12, wherein the mangrove leaves of the species of
10 *Avicennia marina* and *Rhizophora apiculate* or other species of the *Rhizophoraceae* family are in a blending percentage from 0-100% of mangrove species selected.