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Introduction

Probiotics are now offering a promising alternative approach to controlling fish or shrimp diseases and improving the animal health through their ability to control pathogens and enhance nutrition through the development of digestive enzymes. Several routes are used for probiotic administration in aquaculture systems. Probiotics may be administered directly or added to the water as a dietary supplement. Also, the incorporation of probiotics into animal feed pellets is more effective than the direct application of probiotics in rearing systems. This report analyses the viability of the probiotics incorporated in the aqua diet which passes through the extruder or pelleting temperature.

Probiotic organisms such as live bacteria and yeast are likely to have a positive effect on the gut health of animals only if they reach the gut alive. Feed processing conditions, namely moisture addition and exposure to high temperature, shear stress and high pressure have the potential to lower the viability of probiotics. This report addresses a few techniques that enhance the probiotic survival in the extruded pellet diets. However, more research needs to be focused to improve the probiotics viability in pellet diets for the well-being of the aquaculture organisms.

Probiotics

Probiotics are currently gaining scientific and commercial interest, and are now quite common in health promoting therapeutic, prophylactic and growth supplements in functional foods. Studies are focused on microorganism's characteristic of intestinal microbiota for many years, and the term 'probiotic' was primarily restricted to Gram-positive lactic-acid bacteria, especially representative of the genera Bifidobacterium, Lactobacillus and Streptococcus. Unlike terrestrial animals, due to the flow of water passing through the digestive tract, gastrointestinal microbiota of aquatic species is particularly dependent on the outer environment.

Benefits of probiotics in aqua diet

- ❖ The probiotics of Bacillus in aqua diet have shown a positive approach to increasing the size and weight.
- ❖ Immune response stimulation is performed for host bio-security by probiotics applying different biological functions to kill pathogens.
- ❖ The probiotic results of aqua diet demonstrated a high possibility of prevention of stress in aquatic animals.
- ❖ Reduce the level of ammonia in water to ensure healthier output and increased profits.

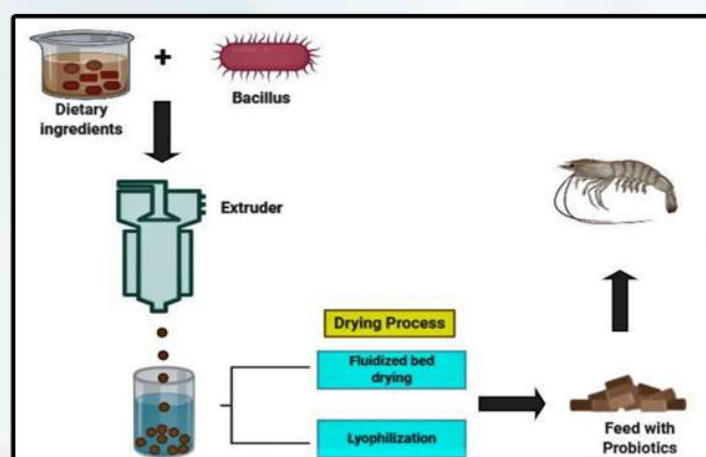


Fig.1. Process of efficient probiotics inoculation in extruder diet for shrimp feeds in aqua diet

Probiotics incorporation into pellet diets

Probiotics viability and stability have been a technological challenge in the production of feed, since probiotics including *Lactobacillus*, *Bacillus* and yeast are susceptible to high temperatures in the pelleting and drying process. *Bacillus* spores in the diet were found to have a loss of more than 99% after the processes of extrusion, extension and drying. Also, the viable yeast count in shrimp feed pellets decreased by 105 fold after being extruded by a meat grinder at 72°C for 31 s, followed by drying at 65°C for 6 h. Some of the methods that help to restore the probiotics in aqua diets (Fig. 1 & Table 1) which are discussed below.

Preparation of shrimp feed with probiotics using fluidized bed dryer method

Fluid bed drying is widely used for the drying of wet particles and granular materials. In a fluidized bed dryer, the probiotic cell suspension is mixed with a vibrating absorber bed or a matrix molecule that helps to form capsules by adhesion. *Lactobacillus lactis* was isolated and cultured overnight in 250 mL Erlenmeyer flasks containing 120 mL of sterile soymilk. For 24 hours, the sample was incubated at 37°C. The formulated feed for shrimps consisted of 40% fishmeal, 8% shrimp head meal, 20% rice bran, 10% wheat flour, 5% sago flour, 10% horse tamarind leaves powder, 5% soybean oil and 2% premix by weight. The mixture was sterilized at 121°C for 30 min and dried overnight in a hot air oven. The culture of the overnight *L.lactis* with and without pH adjustment of 5 M NaOH to 7.0 was added to the mixture. Soybean oil was then applied after 3 minutes of mixing and blended again for 3 minutes. Pressing the feed mixture into pellets at ambient temperature by using a 2 mm diameter pellet mill.

The wet pellets (250 g) were dried at an air inlet temperature of 80°C in a fluidized bed dryer with a 5 L stainless chamber. Milk powder and monosodium glutamate were applied to the overnight culture of *L. lactis*. It had a protective effect on probiotic viability at a high temperature of 80°C. Dried pellets (5 g) of shrimp feed were packed in plastic zip bags and kept at 4°C for 6 months. The viable cell counts in the feed can be measured on monthly using pour plate technique.

Probiotics in low-fishmeal extruded pellet aquafeed

Bacillus licheniformis received attention as probiotic supplements in aquafeed due to the production of heat-stable and low pH resistant spores. The spores also survived the extrusion process during low-fishmeal aquafeed production. Soybean meal (40 to 51% crude protein) with a balanced amino acid profile as well as low cost and market availability is considered to be one of the most promising fish meal substitutes in the aquafeed industry. To achieve a final concentration of 106 CFU/g, *B. licheniformis* was thoroughly mixed with low-fishmeal aquafeed. The mixed feed was then subjected to the extruder with twin-screw. Low-fishmeal EP feed was then air-dried for 3 hours at 60°C followed by vacuum fish oil coating and processed at -20°C before use. The high survival rate of *B. licheniformis* spores at high temperatures at 90°C indicates that it can be used as a food additive.

Conclusion

Research on modern technology that can protect and retain the feasibility of probiotics in aqua diet is needed. Technology should be further developed for the monitoring and maintenance of probiotics survival in the feed. For example, a brief understanding of the chemical composition and activity of the native microorganism and its microbial cultures and molecular knowledge is required to overcome the concept.

PROBIOTICS	EXTRUDER PROCESS	DRYING PROCESS
<i>Bacillus megaterium</i>	An extruder was used for pellet production at temperatures between 70 and 75°C	Pellet was air-dried in a vent hood at room temperature overnight
<i>Bacillus S11</i>	Probiotics were mixed thoroughly with low-fishmeal aquafeed and then subjected to the twin-screw extruder with the following conditions: feeder supply speed, 70 kg/h; conditioner temperature, 80°C	A low-fishmeal feed with probiotics was air-dried at 60°C for 3 h followed by vacuum fish oil coating and stored at -20°C until use
<i>Lactobacillus acidophilus</i>	Probiotics were added to the feed mix after cooling and passed through an extruder, barrel temperature of 60°C and cutter speed of 1100 rpm	Pellets thus obtained were dried at 60°C in an oven and put in airtight polyethylene until their use
<i>Saccharomyces cerevisiae</i>	Diet with yeast was added and mixed until a stiff dough was obtained. This was then extruded through a meat grinder. The pellet temperature just after pelleting was 82°C	After drying in a vertical cooler, the pellets (4mm diameter) were sampled and stored in plastic bags at 4°C
<i>Bacillus subtilis</i>	Extruder at 95°C was applied to the pellets to test the probiotic temperature resistance. Feeds were broken up, sieved to convenient pellet size	Later the fishmeal with probiotic was stored at -20°C

Table 1. Probiotics survival at different temperature during extruder and drying process