



Tributylin as Functional Additive for Fish and Crustaceans Aquafeeds

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How to cite this article:

Kumari, P., Kajal, K., Jana, P., Jayraj, P. and Singh, A. K. 2026. Tributyrin as Functional Additive for Fish and Crustaceans Aquafeeds. *Blue Spectrum*. 1(1): 49-54.

ABSTRACT

Tributylin, a triglyceride of butyric acid and glycerol, has emerged as a promising functional feed additive for sustainable aquaculture. Research in fish and crustaceans demonstrates that dietary tributyrin can improve growth, feed utilization, intestinal morphology, digestive enzyme activity, antioxidant capacity, immune function, and gut microbial composition. Its beneficial effects are particularly relevant in plant-based aquafeeds, where tributyrin may alleviate intestinal dysfunction associated with high levels of soybean meal and other alternative ingredients. Recent studies in largemouth bass, yellow catfish, Pacific white shrimp, and swimming crab further support its functional potential. Nevertheless, responses vary with species, diet composition, and dosage, requiring further research to optimize practical applications.

KEYWORDS

Tributylin, Intestinal health; Growth metrics; Sustainable aquaculture

Introduction

The recent surge in aquaculture production over the past few decades, along with its anticipated expansion in the future, presents a range of challenges for the industry. Recent advancements in fish nutrition research have led to creation of new, balanced commercial diets that significantly enhance fish growth and health (Makode, 2017). It has been observed that formulated feed is crucial in semi-intensive fish farming, as it is necessary to sustain higher fish densities than what natural water fertility can support. Therefore, supplementary feed is regarded as an essential strategy for boosting fish yield (Jhingran, 1991).

The stagnation of marine capture fisheries leads to more dependency on plant-based ingredients which is deficit in limiting amino acid methionine and lysine (Deborde et al., 2021), presence of anti-nutrients and deficiency in long chain polyunsaturated n-3 fatty acid [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)], (Alhazzaa et al., 2019; Cottrell et al., 2020). Plant-based diets can cause increase in inflammation, gut irritation, and intestinal issues, potentially contributing to the development of enteritis (Blaufuss et al., 2020; Palma et al., 2021).

Short-chain fatty acids (SCFA), includes butyric acid and its different derivative sodium butyrate, which is known to promote healing of intestine by increasing the development of epithelial cell and increases function of intestinal absorption (Abdel-Latif

et al., 2020). In formulation of feed for fish and crustaceans, SCFA is a great choice as feed additives which has promising effect in overall health as it balances gut activity, enhance immunity, along with it act as source of energy for cells of gut, anti-inflammatory properties (Tran et al., 2020).

Butyric acid and its derivatives

Butyric acid is a four-carbon odorous volatile, biologically active short chain fatty acid, which can also be produced in gut by bacterial fermentation of dietary carbohydrate including dietary fibre. It is most commonly found in two forms i.e. salt and ester form, known as butyrate and butanoate, respectively (Jiang et al., 2018).

Tributylin (TB), a triglyceride made up of butyric acid and glycerol, is a notable derivative of butyric acid that has been extensively researched and used as a feed supplement for livestock. Its application in aquafeeds, while more recent and less studied, is gaining attention. This growing interest in using dietary tributyrin for fish and crustaceans has prompted the current article, which focuses specifically on species relevant to aquaculture production.

Butyric acid: source

The advantageous effects of butyric acid on metabolism, overall health, and gut microbiota balance have led to increased interest in adding it to feeds for production animals. However, challenges such as its high volatility, strong odour, corrosiveness, and significant absorption in the upper gut can limit its

effectiveness (Bedford and Gong, 2018). Poly- β -hydroxybutyrate (PHB) is a bacterial compound used for energy and carbon storage and has been investigated as an additive in aquafeeds to enhance production in fish and shrimp. Sodium butyrate, a more stable and less odorous salt form of butyric acid, is often added to animal feeds. It can be included directly in diets or encapsulated with triglyceride coatings, depending on whether the goal is to target the small or large intestine (Abdel-Latif et al., 2020). The choice between coated and uncoated forms of butyrate, and the site of its release, should be considered based on the specific goals of supplementation during diet optimization (Ahsan et al., 2016). Conversely, Tributyrin is another butyric acid source frequently used in both human and livestock diets, and its application as a dietary supplement for fish and crustaceans has only recently started to be investigated.

Metabolism and biological function of butyric acid

Plant cell-wall polysaccharides and undigested starches from the small intestine are fermented by anaerobic microbiota in the colon, resulting in the production of short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate (Scheppach et al., 2004). One of the most recognized effects of SCFAs is their

ability to stimulate the proliferation of epithelial cells in the intestine (Scheppach and Weiler, 2004). The mechanisms by which SCFAs promote gut health are still not fully understood and can be complex, warranting further research (Liu et al., 2018). For instance, butyrate has been shown to cross the blood-brain barrier, potentially influencing appetite and eating behaviours. Additionally, butyrate might interact with the gut-brain axis, possibly involving epigenetic mechanisms (Soret et al., 2010).

Tributyrin in aquatic animal feeds

The inclusion of humic acid, citric acid, propionic acid salts, malic acid, formic acid salts (such as potassium diformate), sodium diformate, and various mixtures of short-chain fatty acids (SCFAs) as dietary supplements has produced positive results in several fish species. Nevertheless, butyrate remains the most commonly tested short-chain fatty acid (SCFA) as a supplement in fish diets. In aquaculture species, butyrate is predominantly given as sodium butyrate (Abdel-Latif et al., 2020). Although trials of butyrate in aquatic animals are relatively new, and details of these findings are summarized in Table 2. **Table 2: Dietary supplementation of tributyrin in crustacean species**

Fish species	Dosage (%) (% purity)	Initial weight (g)	Final weight (g)	Effects	References
Pacific White-leg shrimp	0.05% 0.10% 0.15% (98%)	0.88 ± 0.02	8.21 ± 0.52	Tributyrin @ 0.10% resulted in increase	Ding et al. (2022)

(<i>Litopenaeus vannamei</i>)				d SGR, FBW and WG, intestinal digestive enzyme activities,	
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				upregulated gene expression of intestinal antioxidant and immune enzymes, increased abundance of probiotic bacteria, although reduced intestinal microbiota diversity.	
Pacific White-leg shrimp (<i>Litopenaeus vannamei</i>)	0.1%, 0.2% (60%)	0.38 ± 0.01	5.72 ± 0.24	Improved WG, SGR and PER, reduced FCR at 0.2%, increased glutathione peroxidase activity in haemolymph at 0.1% and 0.2%, without changing innate immune parameters; increase in apparent digestibility of lipid and relative mRNA expression levels of IGF-binding protein and prophenol	Lee et al. (2021)

				oxidase genes in hepatopancreas.	
Pacific White-leg shrimp (<i>Litopenaeus vannamei</i>)	1% (Not reported)	1.82 ± 0.01	5.06 ± 0.04	Tributyrin increased zootechnical performance and nutrient retention, elevated trypsin activity, triggered immune response and altered intestinal morphology.	Liu et al. (2021)

Impact of tributyrin supplementation on microbiota of gut content

Dietary tributyrin appears to reshape the gut microbial community in fish, with downstream effects on host physiology. Most of the available research on this topic has looked at tributyrin as a tool for offsetting the drawbacks of plant-heavy feeds, so data are still relatively scarce. One example comes from common carp raised on a plant-based formulation (12% fishmeal, 50% plant meal) and given four different tributyrin doses (0.05%, 0.10%, 0.20%, and 0.40%) over an eight-week trial. By the end of the period, the abundance of Proteobacteria and Actinobacteria followed a dose-dependent pattern: both groups shrank in fish receiving no tributyrin or only the lowest dose (0.05%), but expanded again at the higher doses (0.20% and 0.40%).

A separate eight-week trial in juvenile yellow drum (*Nibea albiflora*) compared a fishmeal-based diet to soybean-meal (SBM) diets supplemented with the same four tributyrin levels. Fish given the SBM diet with no tributyrin showed a drop in gut microbial diversity, which the authors interpreted as a sign of compromised gut health (Tan et al.,

2020). Looking at specific taxa, the unsupplemented SBM group was dominated by *Acinetobacter*, a genus associated with antibiotic resistance and infection risk, over the comparatively benign *Brevundimonas*. That balance reversed in the fishmeal group and in the SBM group receiving 0.10% tributyrin, where *Brevundimonas* became the dominant genus instead. The proposed mechanism is that butyrate released during tributyrin breakdown shifts the local pH, favoring bacteria capable of breaking down and denitrifying organic material — a change that may in turn improve how well the fish digest soy protein (Tan et al., 2020).

Conclusion

Tributylin supplementation in feeds for production animals demonstrates significant potential for enhancing overall animal performance, welfare, and productivity, offering a promising alternative to reduce antimicrobial use in livestock. For aquatic animals, especially fish, tributyrin has been primarily tested in scenarios with high fishmeal replacement and has shown benefits in mitigating some negative impacts, such as gut inflammation, associated with high plant ingredient inclusion. Evaluating different levels of tributyrin across various feed formulations and species could lead to improved aquaculture feeds, optimizing tributyrin's potential to enhance production conditions and animal health. Further research into how tributyrin is metabolized and interacts with gut microbiota will clarify its mechanisms and benefits for fish performance and welfare.

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