

Probiotics in Shrimp Aquaculture: A Systematic Review of Effects on Survival and Disease Resistance

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ABSTRACT

Probiotic bacteria are now routine additions to shrimp feed and pond water, and they are marketed largely on the promise of better survival and stronger resistance to disease. This narrative review asks how firm that promise is. It draws on published work on the main shrimp pathogens, the mechanisms proposed for probiotic action, the experimental challenge studies on which many claims rest, and the literature on safety and regulation, and it reports no new data. Many studies describe benefit, but they differ so widely in strain, dose, route of delivery, host stage and challenge model that their results cannot be pooled in any simple way. Most trials are short, use small tanks and treat tanks or animals as independent replicates, and a literature made up mostly of positive reports invites concern about publication bias. Protection against a laboratory challenge with one bacterial strain says little about a pond in which pathogens, water chemistry and resident microbes all interact, and probiotics have little plausible direct effect on viral disease. Questions of strain identity, gene transfer and regulatory status receive less attention than they deserve. The review concludes that probiotics are a reasonable and probably useful part of shrimp health management but that the size of their effect on survival is unproven, and it sets out what better evidence would look like.

Keywords: Shrimp aquaculture; Probiotics; Survival; Disease resistance; Vibriosis; Challenge models; Narrative review

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Article Class: Narrative Review

1. Introduction

Farmed shrimp is among the most valuable products of aquaculture, and it is also among the most exposed to disease. Intensive culture of the Pacific white shrimp and the black tiger shrimp has repeatedly been interrupted by outbreaks of bacterial and viral disease that spread through ponds and across regions [1, 2]. The newest of these, acute hepatopancreatic necrosis disease, is caused by strains of a common marine bacterium

that carry a plasmid encoding a toxin [3, 4]. For a farmer, the practical result is the same whatever the cause: animals die, often early in the cycle, and the investment in feed and seed is lost.

For many years the response to bacterial disease was antibiotics, applied heavily and often prophylactically [5]. The costs of that habit are now well described, among them selection for resistant bacteria in farm environments and concern about residues and trade [6]. The search

for alternatives has produced a long list of candidates, and probiotics sit near the top of it because they seem to offer protection without the drawbacks [7, 8]. They are widely sold, widely used, and widely written about.

This review asks a plain question about that literature: how strong is the evidence that probiotics improve the survival of cultured shrimp and their resistance to disease? The answer is more complicated than the volume of positive reports suggests. The studies use very different strains, doses and challenge models, many are small tank trials, positive results are more likely to be published than null ones, questions of safety and regulation have received less attention than efficacy, and a benefit seen against a laboratory challenge may not carry over to a production pond. The aim is a fair account of what is known, and of what has merely been repeated often enough to sound known.

The article is a narrative review. It draws on published work on shrimp disease, shrimp immunity and gut microbes, the proposed modes of action of probiotics, challenge experiments, and the literature on safety and on the design and reporting of trials, and it deliberately reports no new data. Where evidence is thin we say so, and we describe what a convincing study would contain instead of quoting figures we cannot check against their sources.

2. Literature Review

2.1 Shrimp Culture and the Disease Problem

Shrimp culture is dominated by a small number of penaeid species grown in earthen ponds, lined ponds and, increasingly, intensive systems with high stocking and heavy feeding. The history of the industry is a history of disease episodes. Viral diseases, of which white spot syndrome is the best known, have caused severe losses in Asia and the Americas, and bacterial diseases, particularly those caused by members of the genus *Vibrio*, are a continuing burden [1, 2]. The emergence of acute hepatopancreatic necrosis disease showed how quickly a bacterial problem can reach the scale of a regional crisis [2, 4].

Vibrio is a large and varied genus, and most of its members are ordinary residents of coastal water and shrimp guts. What separates a harmless strain from a pathogenic one can be as small as a single plasmid [4]. This matters for probiotic work in two ways. A strategy that suppresses vibrios in general may not suppress the particular strain that causes disease, and a strategy that targets one pathogen may do nothing for another. It also means that the

word vibriosis covers a family of conditions, not one, and results obtained with one pathogen cannot be assumed to hold for the rest.

Viral disease raises a separate issue. A bacterium given in feed can compete with, or antagonise, other bacteria, but it has no obvious way of acting directly on a virus. Reports of benefit against viral challenge are therefore usually explained through effects on host immunity or on general condition, which is a more indirect and less certain route [9, 10]. Keeping the distinction between bacterial and viral disease in view prevents a good deal of loose reasoning in the probiotic literature. This is one reason the same product can look excellent in one report and unremarkable in the next.

2.2 Shrimp Immunity and the Gut Community

Shrimp, like other crustaceans, lack the adaptive immune system of vertebrates and depend on innate defences: haemocytes, a melanisation cascade built around the prophenoloxidase system, antimicrobial peptides and a range of recognition and signalling molecules [11, 12]. This has two consequences for probiotic research. First, there is no antibody response to measure, so studies rely on proxies such as enzyme activities and gene expression. Second, the idea of training the immune system in the manner of a vaccine does not transfer directly, although stimulation of innate responses by microbial components is well documented.

The gut is where most probiotics are expected to act. The intestinal community of shrimp differs between wild and domesticated animals [13], and between healthy animals and animals in outbreak conditions [14]. Work on pond-reared shrimp has linked changes in the intestinal community to the onset and severity of disease [15]. These findings are an argument for taking the gut community seriously, but they are associations. Whether a changed community is a cause, a consequence or a bystander of disease is not settled, and that uncertainty runs through the probiotic literature.

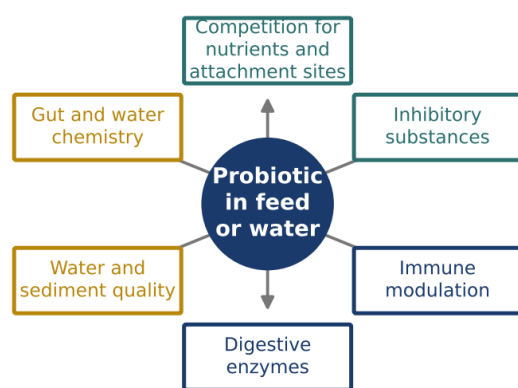
Immunological measurements deserve cautious reading. A higher enzyme activity or a higher transcript level of an immune gene after probiotic feeding is usually reported as an enhancement of immunity, but its link to survival under real conditions is rarely tested directly. Immune activation also has a cost, and a response that helps against one pathogen at one stage may be wasteful or unhelpful at another. A claim about immunity is thus a claim about a surrogate, and surrogates need not predict the outcome that matters to a farmer. A gut that has had time to

settle behaves differently from one that has just met a new bacterium.

2.3 What Probiotics Are and How They Might Work

The word probiotic has a clear meaning in human nutrition, where it refers to live microorganisms that, given in adequate amounts, confer a health benefit [16]. Aquaculture has long used a wider definition that includes microbial additions that improve the water or the pond bottom as well as the host [8, 17]. The wider usage is reasonable for a system in which animals live in their medium, but it has a cost, because products with very different intended actions are placed under one heading and their results are compared as if they were the same thing.

Several modes of action have been proposed (Figure 1). Probiotics may compete with pathogens for nutrients or attachment sites, produce substances that inhibit them, and alter the chemistry of the gut or the water. They may supply enzymes that help digestion, and they may modulate the immune response of the host [8, 18]. In shrimp ponds, there is an additional argument that certain bacteria improve water and sediment quality and so reduce stress [19, 20]. Reviews of the shrimp literature describe all of these as plausible [21, 22, 23].



Schematic of proposed actions; not data

Figure 1. Modes of action proposed for probiotics in shrimp culture (schematic, not data).

Plausible is not the same as demonstrated. Many of the mechanisms have been shown in vitro, for example by the inhibition of *Vibrio* on an agar plate [24, 25], and fewer have been shown in the animal under realistic conditions. A strain that inhibits a pathogen on a plate may not survive the gut, may not reach a useful density there, or may be outcompeted by the resident community. Screening procedures that start from the plate and end in the tank have been criticised for exactly this

reason [26].

2.4 Strains, Delivery and the Culture Cycle

The probiotics studied in shrimp come from several groups of microbes (Table 1). Spore-forming *Bacillus* species are popular because spores tolerate feed processing and storage, and there are reports of *Bacillus* strains protecting black tiger shrimp and Pacific white shrimp against challenge with *Vibrio* [24, 25, 27]. Other trials have used different bacteria, mixtures of strains, or products that combine a bacterium with a prebiotic [9, 28]. The range of candidate organisms is wide, and the range of products on the market is wider.

Delivery also varies. Probiotics can be mixed into feed, which targets the gut, or added to the water, which targets the pond environment and the surfaces of the animal, or both [19, 28]. They can be given from the hatchery onward, or only during grow-out, or only when trouble is expected (Figure 2). Each choice produces a different exposure and a different question for the experimenter. A review that places these under one heading and treats them as a single intervention is combining things that are not the same.

Table 1. Groups of microbes used as probiotics in shrimp culture and the reasons usually given for them.

Group	Typical rationale	Main open question
<i>Bacillus</i> species	Spore formers that survive feed processing and storage; reported to inhibit <i>Vibrio</i> in vitro [24, 25]	Whether activity in the plate predicts activity in the gut [26]
Lactic acid bacteria	Common in fermented foods and the guts of many animals [16]	Persistence in the shrimp gut, which is not a typical lactic acid niche
Yeasts and other microbes	Offered as immune stimulants or nutrient sources	Limited comparative work in shrimp
Strains from the shrimp or its pond	Selected for adaptation to the host and the water [26]	Identity and safety of each strain need documenting [29]
Mixtures of strains	Intended to combine modes of action [41]	Contribution of each member is rarely tested

Note: Qualitative summary of the stated rationale in the cited literature; it is not a ranking of effectiveness.

The culture cycle matters for a further reason. Larvae, postlarvae and juveniles differ in gut structure, immune competence and the microbes they carry, so a result obtained at one stage need not hold at another [22, 26]. Hatcheries and ponds are also managed differently, and a probiotic used to rescue a failing larval run is asked to do a

different job from one fed to juveniles over several weeks to protect them in a pond. Differences of that kind look small in a single tank and matter a great deal across a production cycle.

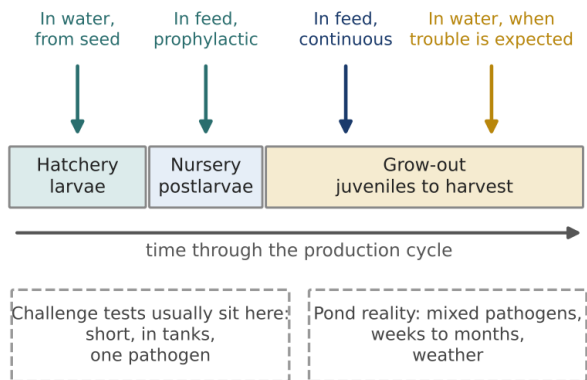


Figure 2. Where probiotics enter the culture cycle and where challenge tests usually sit (schematic timeline, not data).

2.5 Why the Idea Has Appealed

Probiotics appeal for reasons that have as much to do with farming as with biology. They are applied in feed or water with little new equipment, they are seen as natural, and they fit the aim of reducing antibiotic use [5, 7]. In places where regulators or buyers press farmers to cut antibiotics, a probiotic is an easy thing to adopt. These reasons are sound, but they are not evidence of efficacy, and the enthusiasm they generate can leak into the literature in the form of optimistic readings of mixed results.

2.6 Safety and Regulation in Brief

Safety is the least developed part of the literature. Guidance on the safety of probiotics for human use stresses that each strain has to be identified correctly, characterised, and assessed for virulence and for transferable antibiotic resistance genes [29]. Probiotic bacteria can carry resistance genes, and where those genes sit on mobile elements, the product could in principle become a vehicle for their spread [30, 31]. Aquaculture environments are already recognised as places where resistance genes accumulate and move [6].

Regulatory treatment of aquaculture probiotics differs between countries, and in many places products are sold with labels that give a genus name, a count and a list of claims, but little else. A product that carries a species name on the label may not contain what it says, and a strain may be present that has never been assessed. These are not criticisms of any particular manufacturer. They describe a gap between what the efficacy

literature assumes, namely a defined and safe strain, and what a farmer may actually be buying.

3. Materials and Methods

3.1 Scope and Question

The review asks a single question in two parts: do probiotics plausibly improve the survival of cultured shrimp, and do they plausibly increase resistance to disease? The question was divided into the sub-questions that a study would have to answer: which organism was used and how it was identified, how and for how long it was given, at what life stage, against which pathogen and by what route the animals were challenged, how many tanks or ponds were used, and what was measured. None of these distinctions is subtle, but each is easy to lose in a busy hatchery or on a crowded farm.

3.2 Literature Assembly

The review draws on peer-reviewed work in four areas: the biology of shrimp disease and immunity; the proposed mechanisms and the experimental evidence for probiotics in shrimp and other aquatic animals; the safety and regulatory literature; and the wider literature on the design and reporting of experiments. Sources were chosen for relevance, citation in the field and the clarity of their methods. Studies of other species are used for what they teach about mechanisms and limits, not as evidence of effects in shrimp.

Although the title of this article refers to a systematic review, we did not carry out one. We ran no formal search protocol, no registered question, no dual screening and no quantitative synthesis, and we did not follow reporting guidance designed for systematic reviews [32]. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. Narrative reviews have a legitimate place, particularly for mapping a field and for judging how far its evidence can be trusted [33]. The practical consequence is that the review is a better guide to what a good study should contain than to the exact size of any reported effect, and it avoids quoting figures from individual trials that could not be checked against the primary papers.

3.3 Appraisal Framework

To judge how far a report can be trusted, we used a short framework with four questions. Is the intervention defined well enough to be repeated, with the strain identified and the dose and duration stated? Is the challenge model relevant to the disease the product is meant to prevent? Is the design able to support a causal reading, with

enough replicate units and an analysis that treats the right unit as the replicate [34]? And does the body of reports show signs of selective publication or selective reporting [35, 36]? Differences between studies were considered in the manner of heterogeneity in a meta-analysis [37], but without calculating any statistic.

4. Results

4.1 What the Positive Reports Show

A large part of the literature describes benefit. Early work with a *Bacillus* probiont in black tiger shrimp reported better survival and growth and, in a later study, enhancement of immune measures [27, 38]. Subsequent work with *Bacillus subtilis* strains reported inhibition of pathogenic vibrios and protection against vibriosis in tanks [24, 25], and a study in Pacific white shrimp described effects on growth, digestive enzymes, immune gene expression and resistance to disease [39]. In ponds, application of *Bacillus* to the water has been reported to help control luminous vibrios [19].

Against viral disease the reports are more varied in kind. A study of a *Bacillus* strain combined with an oligosaccharide described changes in gut microbial populations, immune responses and resistance to white spot virus [9], and reviews of antiviral use of probiotics in shrimp describe such effects as indirect [10]. It would be a mistake to read these results as showing that a bacterium can neutralise a virus. They show, at most, that some feeding regimes are associated with better outcomes after viral exposure in an experimental setting. The point is not that a trial is poor, only that its result travels less far than its abstract suggests.

4.2 Heterogeneity of Strains, Doses and Challenge Models

The first difficulty is that studies differ in almost every respect (Table 2). Strains differ in genus, species and isolate, and are sometimes not identified beyond a trade name [21, 40]. Doses are expressed in different units and given over different periods [27, 39]. Hosts range from larvae to juveniles, and challenges range from injection of a pathogen to immersion or feeding [3, 9]. Outcomes include survival, growth, enzyme activity and gene expression [38, 39].

Heterogeneity of this size has two consequences. It makes a pooled estimate hard to interpret, because the studies are not estimating one quantity [37]. And it makes disagreement between studies hard to explain. If one strain protects and

another does not, the difference may lie in the strain, the dose, the host stage, the pathogen or the water, and the data seldom allow these to be separated. The reviews that have summarised the area describe the diversity of approaches as a challenge in itself [41, 42].

Table 2. Sources of heterogeneity in probiotic challenge studies in shrimp.

Feature	How studies differ	Why it matters
Strain and species	Different genera, isolates, single strains and mixtures [21, 40]	Results cannot be pooled by genus alone
Dose and duration	Different concentrations, schedules and lengths of feeding [27, 39]	Dose response is seldom described
Route	In feed, in water or both [19, 28]	Exposure of the gut differs
Host stage	Larvae, postlarvae and juveniles [26]	Gut and immunity change with age
Pathogen and route of challenge	Bacterial or viral; injection, immersion or feeding [3, 9]	Severity and relevance of the challenge differ
Outcome	Survival, growth, gene expression, enzyme activity [38, 39]	Surrogate outcomes need not predict survival

Note: Features are those described across the cited studies; no study is singled out and no figures are implied.

There is also little work on dose response. Studies commonly test one or two levels of a probiotic against a control, which cannot show whether more is better, whether there is a plateau, or whether too much does harm. For an additive that is sold by the kilogram, the shape of the response is exactly what a farmer needs to know, and a design that omits it leaves the central economic question unanswered. Read this way, the absence of a significant effect in a small trial is not evidence that there is none.

4.3 Small Tank Trials and Their Statistics

The second difficulty is scale. Many probiotic experiments in shrimp are run in small tanks or aquaria, with a modest number of animals per tank and a small number of tanks per treatment. Small studies are more likely to produce unstable estimates, and when they do reach significance they tend to overstate the effect [36, 43]. This is a general property of underpowered research, and shrimp science is not unusual in having it, but it matters a great deal for a literature in which most of the weight rests on small experiments.

A related problem is the choice of experimental unit. Where animals share a tank, the tank, not the animal, is the unit that received the treatment, and treating each animal as independent inflates the

apparent precision [34]. A trial with a handful of tanks and many animals per tank may look large when it is in fact small. Tanks housed in the same room can also share water supply, temperature and handling, which further weakens the assumption of independence.

Duration is a third limitation. Survival is a cumulative outcome, and a short challenge may capture only the early mortality, whereas the cost of disease to a farmer is realised over weeks. A probiotic that delays death may look protective at the end of a short trial and make no difference to the final outcome in the pond. Without long-term follow-up, delayed and prevented mortality cannot be told apart. A protective effect that disappears when the challenge is made more realistic is not one a farmer would see.

4.4 Publication Bias and Selective Reporting

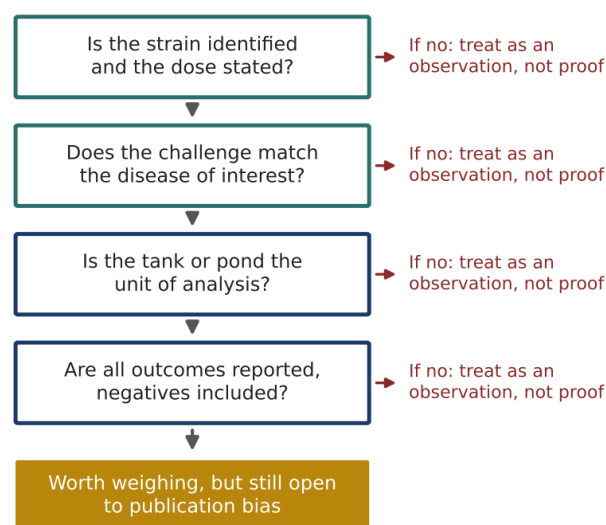
The third difficulty is the likelihood that the published record is more favourable than the full body of experiments. Studies with positive results are more readily written up and accepted than studies with null results, and small studies with chance positive findings are more likely to appear than small studies with chance negative ones [36, 43]. In a field where most published studies are small and most report benefit, this pattern raises a reasonable suspicion of publication bias.

We cannot measure that bias here, because we did not assemble the literature in a way that permits it. In a formal meta-analysis it would be examined through the asymmetry of funnel plots and related tests [35], with the caution that asymmetry can also arise from real differences between small and large studies. The honest statement is narrower: the pattern of the literature is of the kind that tends to be distorted by selective publication, and readers should hold the headline picture of benefit with that in mind.

Selective reporting within studies is a related concern. A trial that measures many outcomes, such as survival, growth, several enzymes and several genes, will find something that differs between groups by chance alone, and a paper may foreground the outcomes that did. Commercial interest in the product is a further, understandable pressure, and several of the reviews that make the field's case are also the ones that call for better standards of evidence [42, 44]. Figure 3 sets out the questions a reader can use to test a claim of benefit.

4.5 Laboratory Challenge Versus Pond Culture

The fourth difficulty concerns what a challenge study measures. A laboratory challenge exposes animals to a defined dose of a defined pathogen by a chosen route, in clean water, in a small container. The infectious nature of a disease such as acute hepatopancreatic necrosis has been established in exactly this way [3], and for that purpose the method is valuable. As a test of a preventive additive, however, it asks a narrow question and answers it in a setting that differs from the farm in nearly every respect (Table 3).



Schematic decision path; not data

Figure 3. Questions to put to a report of probiotic benefit (schematic decision path, not data).

Table 3. Laboratory challenge compared with pond culture as a setting for judging probiotics.

Attribute	Laboratory challenge	Production pond
Pathogen exposure	Defined strain, dose and route [3]	Mixed, variable and often unknown [4]
Water and sediment	Controlled and small in volume	Complex chemistry and large microbial load [19, 20]
Replication	Often few tanks, close together [34]	Few ponds, large differences between them
Outcome	Survival after a set period	Survival, growth, feed use and harvest value
Main risk	Overstated effect, poor transfer	Confounding by management and weather
Strength	Clear causal reading	Real production conditions

Note: Qualitative comparison drawn from the cited literature and the authors' reading of it.

In a pond, exposure to pathogens is continuous, mixed and variable, the water carries a large and

changing microbial load, and the animals are subject to stresses that a tank does not impose [19, 20]. The disease may be caused by a strain that a laboratory challenge never used [4]. A probiotic that suppresses a vibrio in a tank may do little against a pathogen that reaches the animals by a different route, and its effect in the pond may be altered by the microbial community already present. Few of the studies we reviewed tested the same product in both settings.

This does not make tank studies worthless. They give clean causal contrasts and they are the right place to explore mechanisms and screen candidates [26]. The error lies in carrying their results straight to the pond. Field trials, in contrast, have the realism but suffer from few replicate ponds, large differences between ponds and the influence of management and weather. A strong body of evidence would contain both, with each addressing the weakness of the other.

Put plainly, the literature supports only a cautious statement. Probiotics are plausible aids to shrimp health, many experiments report benefit, and some of the proposed mechanisms are well founded. The size of any effect on survival under production conditions is not established by evidence we can cite with confidence, and the best summary is that it is likely to be positive and modest in some circumstances, uncertain in others, and dependent on the strain, the pathogen and the management of the farm. That is a modest claim, and it is the one the evidence will currently bear.

5. Discussion

5.1 Interpretation

The main finding of this review is a conditional one. The body of work gives reasons to expect that some probiotic regimes help shrimp resist some bacterial diseases, but it does not allow a confident estimate of how much, for which products, or under what conditions. That fits what is known more generally about the field, in which reviewers describe both the promise of probiotics and the need for sturdier evidence [40, 42, 44]. Nothing in this reading argues against using probiotics; it argues for measuring what happens when they are used.

The second conditional concerns the type of disease. For bacterial disease there is a direct and testable mechanism, namely competition with or inhibition of the pathogen, even if testing it in the field is hard. For viral disease the route is indirect and the evidence weaker [9, 10]. A farmer facing viral risk should not be led to expect that a

probiotic offers protection of the kind a vaccine offers, because the biology of shrimp immunity does not support that expectation [11, 12].

Three findings would change this reading. A set of well-replicated pond trials with identified strains and fully reported methods showing consistent protection would strengthen the case considerably. A systematic review with a registered protocol that found little difference between treated and untreated animals after allowing for bias would weaken it. And evidence of transferable resistance genes in widely sold products would require the safety question to move to the front of any advice [30, 31]. These points apply with equal force to any new additive, not only to probiotics.

5.2 Limitations

This is a narrative review and shares the limitations of that form. The selection of sources reflects judgement, no formal search or quality scoring was done, and the review does not quote numerical results from individual trials, because these could not be checked against the primary reports for this article. A reader who needs an effect size should consult the original studies directly, applying the appraisal questions above, or wait for a formal systematic review with meta-analysis of the kind that reporting standards exist to support [32, 37].

The review also draws on evidence from other species and from in vitro work, which are imperfect proxies for farmed shrimp. Studies of fish and of human probiotics inform the discussion of mechanisms and safety, but the gut and immune system of a crustacean differ from theirs. We have used them to frame hypotheses, and the conclusions should be read in that light. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5.3 Safety, Strain Identity and Regulation

The safety questions deserve more attention than they have had. At a minimum, a product used in food animals should state the species and strain it contains, be free of known pathogens, and not carry transferable resistance genes [29, 30]. In practice, the efficacy literature seldom reports the checks that would show this, and a reader often cannot tell whether the strain used in a trial is the strain sold to farmers. A good result with an uncharacterised strain provides little assurance about a product with the same name.

The wider context adds weight. Aquaculture is already a recognised reservoir and a route of spread for resistance genes [6], and it would be

unfortunate if a measure introduced to reduce antibiotic use added to that burden. The tension is a real one: probiotics are promoted as a way of limiting resistance, and they can themselves carry resistance determinants unless they are screened [30, 31]. Screening is neither difficult nor costly relative to the value of the crop, and the regulatory systems of producing countries could reasonably require it.

5.4 Directions for Future Work

A trial that could settle the question would use identified strains deposited in a culture collection, report the dose and the viable count at the time of delivery, and include more than one dose. It would use enough replicate tanks or ponds to detect a plausible effect, analyse at the level at which the treatment was applied [34], and report effects as estimates with intervals instead of only significance. It would follow animals to harvest, and it would record survival, growth and feed use as the outcomes that matter to producers.

Beyond single trials, three needs stand out. A registered systematic review and meta-analysis, with a transparent search, a defined appraisal of bias and a test for small-study effects, would show how much of the apparent benefit survives [32, 35]. Field trials that compare laboratory-validated products in production ponds, with the same product tested in both settings, would address the question of transfer. And work on the safety and identity of commercial products would close the gap described in the preceding section.

6. Conclusion

Probiotics are a reasonable and probably useful part of shrimp health management. They are biologically plausible, they have been reported to help against bacterial disease in a range of experiments, and they offer an alternative to routine antibiotic use. What the literature does not yet establish is how large their effect on survival and disease resistance is on a working farm, because studies differ widely in strain, dose and challenge model, are often small tank experiments, probably reflect some publication bias, and have mostly been run under laboratory conditions that differ from ponds.

For the practitioner the message is practical rather than discouraging. Probiotics can be tried, provided the product is identified and screened, the claims are treated with care, and the farm keeps records that allow its own results to be judged. For the researcher, the opportunity is a set of properly replicated, fully reported studies, in

tanks and in ponds, that turn a plausible idea into a measured result on which farmers and regulators can rely.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

S.F. conceived the review and wrote the sections on shrimp disease and health management. K.V. wrote the sections on culture systems, probiotic delivery and challenge models. O.B. wrote the sections on safety, regulation and evidence appraisal. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No animals were used and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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